Effect of D-allulose, in comparison to sucrose and D-fructose, on the physical properties of cupcakes

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Alexandra May Bolger, Robert A Rastall, Maria Jose Oruna-Concha, Julia Rodriguez Garcia*

Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading, UK, RG6 6DZ

* Corresponding author. *Email address*: j.rodriguezgarcia@reading.ac.uk; *Telephone*: +44 (0)118-378-4734

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Highlights

- D-allulose did not retard the onset of starch gelatinisation as effectively as sucrose did.
- Weight loss during baking was slower for D-allulose cupcakes.
- Textures of D-allulose and sucrose cupcakes were comparable at the longest baking time.
- D-allulose and D-fructose produced cupcakes of similar physical properties.

Abstract

The high sucrose content in sweet bakery products is a nutritional concern. However, the technological functions of sucrose in such products make its replacement challenging. D-allulose, which only has 5% of the energy content of sucrose, may be an ideal sucrose substitute, due to its similar physicochemical and bulk properties. The aim of this study was to investigate the performance of D-allulose as the main sugar component in cupcakes, in comparison to both sucrose and D-fructose, by assessing the physicochemical and physical properties of cupcakes baked for 12, 16, and 20 min. D-allulose batters experienced less volume expansion and took longer to lose weight and thermally set during baking than did sucrose batters. D-allulose cupcakes had lower water activity values at all baking times and developed a browner colour and greater acrylamide concentration, compared to sucrose cupcakes. Comparable textural properties were achieved by D-allulose and sucrose cupcakes at the longest baking time (20 min). D-allulose and D-fructose produced cupcakes with comparable physical properties. There is potential for the use of D-allulose in the bakery products industry, by employing longer baking processes, or in other products requiring lower moisture content and less volume development.

Keywords:

D-allulose, sucrose, D-fructose, sugar replacement, cupcake

1. Introduction

Sucrose is a non-reducing disaccharide used worldwide, and this sugar is a principal ingredient of many food products. However, due to the high energy content of sucrose (4 kcal/g), a high intake of this sugar leads to excessive energy consumption. This has been found to be a risk factor for obesity, which has been linked to chronic noncommunicable diseases such as type 2 diabetes and cardiovascular disease. Dietary recommendations by governments and health organisations around the world suggest to limit free sugar intake, from disaccharides (e.g. sucrose) and monosaccharides (e.g. fructose and glucose), which are widely added to foods, to no more than 10% to 5% of total daily energy intake SACN (2015); (WHO, 2015). Consequently, the food industry is being pressured to reformulate products containing high free sugars to address these recommendations.

D-allulose, also known as D-psicose, is a ketohexose monosaccharide and the C-3 epimer of D-fructose. It has a sweetness equivalent to 70% of that of sucrose, but is classed as an ultra-low-calorie sugar because it has an available energy content of just 0.2 kcal/g, meaning it only has 5% of the energy of sucrose or D-fructose (Lamothe et al., 2019). D-allulose also has almost no effect on blood glucose levels, due to its negligible glycaemic index, unlike those of sucrose and D-fructose (Lamothe et al., 2019; Pocan, Ilhan, & Oztop, 2019). Therefore, D-allulose is suitable for consumption by diabetic patients, making it a potentially ideal substitute for sucrose and a better option compared to other free sugars (such as D-fructose), from a nutritional point of view. D-allulose exists in nature only in small quantities and in residue of fermented molasses (Belitz, Grosch, & Schieberle, 2004). However, mass production of D-allulose has become feasible. The introduction of a new technique utilising immobilised enzymes means that D-allulose can be produced from D-fructose by the action of D-tagatose 3-epimerase in a simple yet cost effective method

(Granström, Takata, Tokuda, & Izumori, 2004). The US Food and Drug Administration (FDA) approved D-allulose to be 'generally recognised as safe'(GRAS) (Zhang, Zhang, Jiang, & Mu, 2017). D-allulose is exempted from "Sugars" and "Added Sugars" labelling; and it is now available in a non-GM crystalline form as DOLCIA PRIMA® Crystalline Allulose from Tate & Lyle (Tate & Lyle, 2021).Directional prices in the US market for sucrose, allulose and fructose are $0.94/kg, $3.42/kg and $1.10/kg respectively.

Cakes, cake bars, and other sweet baked goods such as cookies are high-sugar products relished by consumers. Sucrose is the most commonly used sugar in cake making (Struck, Jaros, Brennan, & Rohm, 2014), and it has a variety of functions, besides simply imparting sweetness (E. J. Lee, Moon, & Kweon, 2020; Slade, Kweon, & Levine, 2021). During batter preparation, sucrose helps to form a stable foam-emulsion and minimise gluten development, giving cake its tender texture (Slade et al., 2021; Wilderjans, Luyts, Brijs, & Delcour, 2013) During baking, sucrose elevates phase transitions like starch gelatinisation, gluten and egg white protein thermosetting (Donovan, 1977; Slade & Levine, 1991; van der Sman & Renzetti, 2020). At high temperatures during baking, sucrose inverts to its constituent reducing sugars, D-glucose and D-fructose, which then take part in Maillard and caramelisation reactions, thus providing cake with a pleasant flavour and golden-brown colour (Davis, 1995). Sucrose interacts with and holds water, lowering water activity which limits microbiological activity and mould formation (Davis, 1995; Slade & Levine, 1991); delaying moisture migration during storage, and interacting with starch, thereby delaying the onset of starch retrogradation (Slade et al., 2021; Slade & Levine, 1991), and thus maintaining the moist and soft texture of cake for a longer time (Gélinas, Roy, & Guillet, 1999).

Despite sucrose's extensive list of complex functions, the physicochemical properties of D-allulose suggest that this sugar could be a promising substitute in sweet baked goods such as cookies (Kweon, Slade, Levine, & Gannon, 2014; Young, S., & Kweon, 2016) and cake products (E. J. Lee et al., 2020; Sun, Hayakawa, Ogawa, Fukada, & Izumori, 2008). D-allulose has a high solubility in water, which makes its use in food processing easier, and it also contributes a similar amount of bulk to foods as does sucrose, as well as having a clean taste, smooth texture, and desirable mouthfeel (Sun et al., 2008). D-allulose has also been shown to have an ability to improve product quality and processing characteristics of various foods; it enhanced brown colour development in cookies, as a result of accelerated Maillard reactions, due to its reducing nature (Sun et al., 2008). It has also been shown to improve the foaming capacity (O'Charoen, Hayakawa, Matsumoto, & Ogawa, 2014) and gelling behaviour (Sun, Hayakawa, & Izumori, 2004) of egg white proteins, as well as to improve the textural properties of soft candies (Pocan et al., 2019). A study by P. Lee, Oh, Kim, and Kim (2020) concluded that 25% substitution of sucrose with D-allulose is likely to be appropriate in pound cakes. Although it is worth considering sucrose replacement with D-allulose in cakes and cookies, for nutritionally beneficial reasons, it is still not fully understood how this monosaccharide interacts in such sweet bakery matrices, and how it influences the development of the final crumb structure. Understanding of these mechanisms is essential to allow a more tailored use of D-allulose in such sweet bakery systems, to achieve optimal product physical properties.

The main aim of the present study was to investigate the performance of D-allulose as the main sugar component in cupcakes, in terms of its effect on the thermal setting mechanism of batter and the physical properties of cupcakes, compared to those of sucrose. To support this work, the relationship between ketohexose chemical structure and

technological functionality was investigated by also comparing the performance of D-fructose in the cupcake system. The influence these three sugars had on starch gelatinisation was assessed using differential scanning calorimetry (DSC). All three sugars were then incorporated into cupcake batter, and the resulting cupcakes' weight loss during baking, height, cellular crumb structure, textural properties, colour, and acrylamide levels were each analysed after 3 different baking times (12, 16, and 20 min). The results of this study should provide essential findings for the development of recipes using D-allulose to produce nutritionally improved sweet bakery products such as cakes and cookies that will as well have optimal texture and volume.

2. Methods and Materials

2.1. Ingredients

The ingredients used in this study included the following: sucrose (Caster Sugar, Tate and Lyle, London, UK), D-allulose (Sensato, Netrition Inc., Albany, NY), D-fructose (Danisco, Copenhagen, Denmark), wheat flour (British Plain Flour, protein content: 9.7%; , Sainsburys Supermarkets Ltd, London, UK; specification details supplied by UK Flour Millers Association for UK plain flour :moisture : 13.6 – 14.8 %; colour tristimulus (L*-b*): 77.0 min; Hagberg Falling Number: 150 min; water absorption: 54.0 – 58.0%), margarine (Stork Original Spread, Stork UK and Ireland, London, UK), pasteurised free range whole eggs (West Horsley Dairy Ltd, Woking, UK), baking powder (diphosphates and sodium carbonates)(Dr Oetker (UK) Ltd., Leeds, UK), salt (NaCl) , and distilled water (pH 5.38 (0.10)). Acrylamide (>99%) was purchased from Sigma-Aldrich (St Louis, MO). 1,2,3-^{13}C3-Acrylamide (1000 mg/L in methanol) was purchased from LGC Standards (Teddington, UK); 1 mL was diluted to 1 L in deionized

119 water to provide a 1 mg/L stock solution. Further details on the chemical properties of the
120 sugars used are presented in Table 1.

121 2.2. DSC of Wheat Flour and Sugar Solution Mixtures

122 DSC was used to analyse the thermal gelatinisation behaviour of the starch in wheat flour, in
123 water and in the presence of the three different sugar solutions. A modified version of the
124 method described by Kweon, Slade, and Levine (2016) was followed. Equal mass of wheat
125 flour (wet basis) and 50 g/100 g pre-dissolved sugar solutions were mixed by hand with a
126 spatula to form a homogeneous paste, and then allowed to stand at room temperature
127 overnight. Pre-dissolved sugar solutions were used to avoid the endothermic peak caused by
128 the dissolution of sugar during heating. Approximately 35-40 mg of each 3-component
129 mixture were transferred to a T-zero aluminium DSC pan and hermetically sealed. Each
130 sample was heated in the DSC instrument (TA-Q2000 DSC, TA Instruments, New Caste,
131 Germany) from 30 °C to 130 °C with a 10 K/min heating rate. An empty DSC pan was used as
132 a reference. Calibration was performed using indium and sapphire. Onset, peak, and end
133 gelatinisation temperatures were calculated by analysing the glass transition of the starch
134 (Slade & Levine, 1991) . Gelatinisation enthalpy was calculated by integrating the exothermic
135 peak. Both analyses were conducted using TA Instruments Universal Analysis 2000 Software
136 (version 4.5A). Three replicates were performed on each sugar-containing sample (sucrose,
137 D-allulose, and D-fructose).

138 2.3. Preparation of Cupcake Batter and Baking Procedure

139 Cupcake batters were prepared using a multi-stage mixing method in a free-standing electric
140 mixer (Chef XL KVL4100W, Kenwood, Havant, UK) with a K beater attachment. The recipe for
141 pound cake described by Wilderjans, Pareyt, Goesaert, Brijs, and Delcour (2008) was

142 followed. Ingredient quantities are summarised in Table 2, no water was added as an
143 ingredient in batter formulation. Each batter was prepared by creaming the margarine and
144 one of the three sugars (sucrose, D-allulose, or D-fructose) in the mixer on speed 4 (central
145 rotation: 130rpm and attachment rotation: 315 rpm) for 3 min. Liquid whole egg was then
146 added and mixed on speed 3 (central rotation: 115 rpm and attachment rotation: 270 rpm)
147 for 30 s. Finally, flour, salt, and baking powder were added and mixed on speed 3 for 3 min.
148 Each section of a 12-hole cupcake tray was lined with a paper case and filled with batter to a
149 weight of 50.5 g ± 0.1 g. Each individual cupcake tray was then placed in the centre of a pre-
150 heated oven (Stratos 3STA 4676 H18 S.207, Polin, Verona, Italy), set to 180 °C with a heat
151 distribution of 40%:60% (top:bottom), and baked for the selected time (12 min, 16 min, and
152 20 min). Cupcakes were cooled for 1 h at room temperature, then placed in polyethylene
153 bags, and stored at room temperature for subsequent analysis.

154 A total of 9 cupcake formulations was assessed by combining each of the 3 sugars (sucrose:
155 S; allulose: A; fructose: F) with each of the 3 baking times (12 min: 12; 16 min: 16; 20 min:
156 20). These 9 samples were named with the initial of the sugar used, followed by the number
157 of the baking time applied; for example: sucrose sample baked for 12 min = S12. All samples
158 were prepared in duplicate.

159 2.4. Water Activity of Sugar Solutions and Cupcake Crumb

160 Equal weights of water and sugar were mixed by hand with a spatula to create a 50 g/100 g
161 sugar solution, and then allowed to stand at room temperature overnight. Approximately 10
162 ml of the fully dissolved sugar solution were pipetted into the sample cup of an a_w-meter
163 (HygroLab C1, Rotronic AG, Basserdorf, Switzerland), and water activity was measured at

room temperature (20 °C) using the 'AwQuick' setting. Three replicates were performed on each sugar (sucrose, D-allulose, and D-fructose).

The cupcakes were milled to a fine crumb and mixed. A fine layer of milled crumb was added into the sample cup of the a_w-meter, and water activity was measured at room temperature (20 °C) using the 'AwQuick' setting. Measurements were performed in triplicate.

2.5. Apparent Viscosity of batters

The apparent viscosity of the cupcake batters was measured following Tsatsaragkou, Methven, Chatzifragkou, and Rodriguez-Garcia (2021) method with some modifications. An oscillatory rheometer (MCR 302, Anton Paar Ltd., St Albans, UK) was used. Cupcake batters with no leavening agent were allowed to rest in the measuring cell for 15 min equilibration time at 25 °C. Parallel serrated plate geometry (50 mm diameter; profile 1 mm x 0.5 mm) was employed. Apparent viscosity was measured as a function of shear rate over the 0.1 to 100 s-1 range, at 25 °C. Data were fitted to the following power law (Ostwald model):

$$\eta = K \cdot \gamma^{(n-1)}$$

where η is the viscosity (Pa·s), γ is the shear rate (s−1), K is the consistency index (Pa·sn), and *n* is the flow behaviour index. Two batches of each cupcake batter were analysed.

2.6. Weight Loss During Baking

The weight loss during baking was calculated for each cupcake using the following equation (Martínez-Cervera, Salvador, Muguerza, Moulay, & Fiszman, 2011):

$$W_{loss}(\%) = \left[\frac{W_{batter+case} - W_{cake+case}}{W_{batter+case}} \right] \times 100$$

where W_{loss} is the percentage weight loss during baking, $W_{batter+case}$ is the weight (in grams) of batter in its case before baking, and $W_{cake+case}$ is the weight (in grams) of the cupcake in its case after baking and cooling.

2.7. Cupcake Height

Four cupcakes were cut in half, on a plane perpendicular to their base (vertical axis), with a stainless-steel knife and scanned (HP Scanjet G2710, HP Inc., Palo Alto, CA, USA). The maximum cupcake centre height was measured from the scanned image, using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.8. Cellular Structure of the Cupcake Crumb

Each of the four images used to measure cupcake height was cropped, to a 5 x 3 cm section from the central area of the cupcake, for crumb cell analysis with the ImageJ software (National Institutes of Health, Bethesda, MD, USA). First, the image colour channels were split to access the blue channel. The contrast was then enhanced by 0.4%, and finally the image was binarized after grayscale threshold. Average cell size (in mm^2) and total cell area within the crumb (in percent) were calculated (Rodríguez-García, Sahi, & Hernando, 2014).

2.9. Cupcake Texture

For the evaluation of cupcake crumb texture, a cylindrical piece of crumb (4 cm diameter, 2 cm height) was taken from the centre of each of four cupcakes, with the aid of a stainless-steel cutter. Texture measurements were carried out using a Texture Analyser (TA-XT2, Plus-Upgrade, Stable Micro Systems, Godalming, UK) coupled with Exponent Connect Lite software (version 6.1.16.0, Stable Microsystems). The instrument was equipped with a 5 kg load cell and a cylindrical 75 cm diameter compression probe attachment (SMS P/75). A

double compression test was performed with a test speed of 1 mm/s, and the maximum deformation was 40% of the original cylinder height. The interval between two successive compression cycles was 5 s, and a trigger force of 5 g was selected. From the force-time curves of the Texture Profile Analysis, the parameters of hardness (maximum peak force during the first compression cycle), springiness (distance of the detected height during the second compression divided by the original compression distance), and cohesiveness (ratio of the positive force area during the second compression to that during the first compression) were calculated by the software Exponent (version 6.1.18.0, Stable Micro Systems, UK).

2.10. Cupcake Colour

The colour of both the crumb and crust of four cupcakes (per sugar) was measured using a colorimeter (Chromameter CR-400, Konica Minolta, Tokyo, Japan). The results were expressed in accordance with the CIELAB system (illuminant C and 10° viewing angle). The parameters measured were L* [indicates lightness and ranges from 0 (for black) to 100 (for white)], a* [ranges from -a* (for greenness) to +a* (for redness)], and b* [ranges from -b* (for blueness) to +b* (for yellowness)] (Konica Minolta, 2007; Wu & Sun, 2013). The colorimeter was standardised with a white plate, and the colour of the crust was measured at the centre point of the cupcake top. The cupcake was then cut in half on a plane parallel to its base, and the colour of the crumb was measured at the centre point of the cut surface.

The browning index (BI), which represents the purity of brown colour, was calculated for cupcake crust, as follows (Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, & Swanson, 1999):

$$BI = \frac{100(x - 0.31)}{0.172}$$

where:

$$x = \frac{a^* + 1.75L^*}{5.645L^* * + a^* - 0.3012b^*}$$

Total colour difference (ΔE*) values were calculated for the crust and crumb of cupcakes baked for the same amount of time, in comparison to the sucrose samples (S12, S16, and S20). ΔE* was calculated according to the following equation (Francis & Clydesdale, 1975):

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

The values used, to determine whether total colour differences between cupcakes were appreciable by the human eye, were the following (Bodart, de Peñaranda, Deneyer, & Flamant, 2008):

ΔE* < 1 colour differences are not obvious to the human eye.

1 < ΔE* < 3 colour differences are not appreciated by the human eye.

ΔE* > 3 colour differences are obvious to the human eye.

2.11. Asparagine and Acrylamide Analyses

Asparagine and acrylamide were measured using the method described by Halford et al. (2012). A flour sample (0.100 ± 0.005 g) was weighed into a 14 mL screw-top bottle. Hydrochloric acid (10 mL, 0.01 mol/L) was added to the vial, and the sample was stirred for 15 min at room temperature. An aliquot of supernatant (2 mL) was centrifuged at 10000g for 10 min. The amino acids in 100 µL of the centrifuged supernatant were then derivatized using the EZ-Faast amino acid derivatization technique for GC-MS (Phenomenex, Torrance, CA, USA). GC-MS analysis of the derivatized sample was carried out using an Agilent 5975

249 system (Agilent, Santa Clara, CA, USA). Three replicates were performed for UK plain flour.
250 Asparagine was quantified using a external calibration curve, prepared using a derivatized
251 amino acid standard.

252 For acrylamide extraction milled cupcake samples (1.00 ± 0.01 g) were weighed into 50 ml
253 Falcon tubes. Samples were extracted with 40 ml of deionised water containing 2 ml of
254 internal standard (1,2,3-$^{13}C_3$-acrylamide 1 mg/L) at room temperature. Each sample was
255 shaken in a mechanical shaker (Hei-MIX Multi Reax, Heidolph Instruments, Schwabach,
256 Germany) for 20 min at 1800 rpm, and then centrifuged (Sigma 3k10, Osterode am Harz,
257 Germany) at 15 °C and 9000 rpm for 10 min. Two mL were removed from the aqueous layer
258 and passed through a 0.22-µm pore size syringe filter into a 2-mL vial. All samples were
259 extracted and analysed in triplicate.

260 Cupcake crumb samples were analysed by liquid chromatography-mass spectrometry/mass
261 spectrometry (LC-MS/MS), using an Agilent 1200 High-Performance Liquid Chromatography
262 (HPLC) system, connected to a 6410 triple-quadruple mass spectrometer with electrospray
263 ion source in positive ion mode (both Agilent Technologies, Santa Clara, CA). The mobile
264 phase was 0.1% aqueous formic acid at a flow rate of 0.3 mL/min. The injection volume was
265 5 µL. An isocratic separation was carried out at room temperature, using a 100 x 3.0 mm
266 Hypercarb column with a 10 x 3.0 mm Hypercarb pre-column (both 5 µm particle size;
267 Thermo Fisher Scientific, Waltham, MA). The transitions m/z 72→55 and m/z 72→27 were
268 measured for acrylamide, and the transition m/z 75→58 was measured for $^{13}C_3$-acrylamide.
269 Concentrations of acrylamide in cupcake crumb samples were expressed as µg/kg fresh
270 weight, calculated from a calibration curve prepared from solutions in water containing 1

µg/L, 2.5 µg/L, 5 µg/L, 10 µg/L, 25 µg/L, and 50 µg/L acrylamide, alongside 50 ug/L $^{13}C_3$-acrylamide .

2.12. Statistical Analysis

Statistical analysis of the data was carried out using an XLSTAT (2020) software package (Addinsoft, France). The effect of sugar type on water activity, DSC starch gelatinisation behaviour, batter consistency index and flow behaviour index was determined by one-way ANOVA. The effect of sugar type and baking time on cupcake properties (water activity, weight loss during baking, cell crumb structure, texture, height, colour and acrylamide content) was determined by two-way ANOVA. Multiple pairwise comparisons were performed using Tukey's Honest Significant Difference (HSD) to evaluate mean value differences ($p<0.05$).

3. Results and Discussion

3.1 Starch Gelatinisation

Figure 1 shows that the DSC thermograms of wheat flour samples with the different sugars had the melting transition at higher temperatures than the wheat flour and water sample. The DSC results (Table 3) also showed that sucrose was able to shift starch gelatinisation to higher temperatures than D-allulose and D-fructose. The sucrose solution-wheat flour sample had significantly higher onset, peak, and end gelatinisation temperatures ($p<0.05$), compared to those of the D-fructose solution-wheat flour and D-allulose solution-wheat flour samples, in good agreement with previously reported results (E. J. Lee et al., 2020; Slade & Levine, 1991; Young et al., 2016). Several hypotheses have been proposed to explain the influence different sugars have on the gelatinisation temperature of wheat starch. One of the early theories, as reviewed by Slade and Levine (1991), attributes the effect sucrose

has on reducing water activity to its ability to retard wheat starch gelatinisation, through limiting the availability of water to starch (Derby, Miller, Miller, & Trimbo, 1975) and thus delaying granule swelling. However, if water activity were the factor controlling starch gelatinisation temperature, it would have been expected, based on our DSC results, that a sucrose solution would have a lower water activity than a solution of D-allulose of the same concentration. However, the opposite trend was observed (Table 3); the water activity of the 50 g/100 g D-allulose solution was significantly lower ($p<0.05$) than that of the 50 g/100 g sucrose solution. However, the DSC results in Table 3 could be explained by an alternative theory, whereby the effectiveness of water or an aqueous sugar solution, acting as a plasticiser, in lowering the glass transition temperature (Tg) of the amorphous regions of starch, which represents the beginning of the starch gelatinisation process and thereby precedes and controls the subsequent melting of starch crystalline regions, is determined by the weight-average molecular weight of the sugar-water solvent, and thus follows the order water > monosaccharide > disaccharide, such that the resulting starch gelatinisation peak temperature in water or sugar solution, which follows immediately after the onset Tg of the plasticised amorphous starch, follows the order water < monosaccharide (e.g. D-allulose and D-fructose) < disaccharide (e.g. sucrose) (Slade & Levine, 1991). Moreover, studies by Renzetti, van den Hoek, and van der Sman (2020, 2021); van der Sman and Mauer (2019) have demonstrated that the elevation of melting (phase transition) temperatures of biopolymers, such as egg protein and starch, in the presence of sugars, is a function of the volumetric density of intermolecular hydrogen bonds between water-sugar solution and the biopolymer. Differences in molar volume (V) and effective numbers of hydroxyl groups available for intermolecular hydrogen bonding ($N_{OH,s}$) define the volumetric density of hydrogen bonding sites available in solution (sugar solution) for interactions with starch

($\Phi_{w,eff}$) (Renzetti et al., 2020, 2021). Sucrose has a higher molar volume and effective number of hydroxyl groups available for intermolecular hydrogen bonding ($N_{OH,s}$) (210.2 cm^3/mol, 4.48, respectively) than fructose (110.4 cm^3/mol, 3.98, respectively) (Allan, Chamberlain, & Mauer, 2020; Renzetti et al., 2020) (the authors could not find data on D-allulose molar volume and $N_{OH,s}$). Thus, it has been hypothesised that, after these sugar solutions penetrate the amorphous phase of the starch granules, the disaccharide solution would form more hydrogen bonds with the amorphous phase and thereby increase the melting point of the crystalline regions of the starch to a greater extent than would the monosaccharide solutions (Allan, Rajwa, & Mauer, 2018; van der Sman & Mauer, 2019). These theories could explain why D-allulose and D-fructose, monosaccharides, were not able to increase the starch gelatinisation temperature as effectively as did sucrose.

Although our study did not examine the thermal setting process involving the denaturation of egg protein, previous research has demonstrated that $\Phi_{w,eff}$ also controls this phase transition (Renzetti et al., 2020). Slade et al. (2021) have previously analysed by DSC, with regard to 1:1 w/w samples of egg white protein in water alone or concentrated sucrose-water solutions. The thermogram curves showed that as the sucrose solution concentration increased, the denaturation peak for ovalbumin increased (Slade et al., 2021). Moreover, it has been demonstrated that at intermediate sugar concentrations (37-60%), close to a cake system, and when the number of effective hydroxyl groups per molar volume of the plasticisers ($N_{OH,}s/v_s$) increase, a separation of the ternary system protein/water/plasticiser occurs, resulting in a biopolymer-rich phase and a plasticiser-rich phase, with water partitioning between the two domains; this gives place to a reduction in the effective hydrogen bond density in the protein-rich domain, resulting in an elevation of the denaturation temperature (Renzetti et al., 2020, 2021). Low molecular weight plasticiser

have $N_{OH,S}/v_s > 29$ and high molecular weight plasticiser have $N_{OH,S}/v_s < 10$. Therefore, it can be hypothesised that D-allulose and D-fructose will increase the denaturation temperature of egg white protein by phase separation in cake-type systems. This mechanism has not been proven in starch gelatinisation (Renzetti et al., 2021).

Although both D-allulose and D-fructose are monosaccharides with the same molecular weight, the D-fructose solution-wheat flour sample had significantly higher onset and peak gelatinisation temperatures ($p<0.05$), compared to those for the D-allulose solution-wheat flour sample, so that D-allulose was the least effective of the three sugars at retarding starch gelatinisation. The reason for this difference might lie in the structural forms these sugars can assume, once dissolved in solution. When sugar molecules are dissolved in aqueous solutions, a series of reactions involving molecular rearrangements takes places around carbon 1, leading to the formation of a mixture of products that are in equilibrium; this is called mutarotation (Cui, 2055). A study by Que and Gray (1974) found that, at equilibrium, in aqueous solutions at 30°C, D-fructose exists mainly in the β-pyranose ring form, whereas the majority of D-allulose exists in the α-furanose ring form. Therefore, based on the sugar-starch interaction theory mentioned above, it could be hypothesised that the β-pyranose ring structure of D-fructose forms stronger bonds with the amorphous regions of starch, compared to those formed by the α-furanose ring structure of D-allulose. However, such an explanation for the observed order of these sugar solutions in retarding wheat starch gelatinisation is only speculation, so further investigation would be required to support it. Alternatively, to distinguish between sugars of equal molecular size and weight (e.g. monosaccharides such as D-allulose and D-fructose), with regard to their effect on starch gelatinisation temperature, Slade and Levine (1991) proposed that the Tg of the sugar could be the determining factor. In the present case, the order of increasing starch

366 gelatinisation temperatures measured for the 3-component mixtures listed in Table 3, and
367 likewise previously reported by E. J. Lee et al. (2020) and Young et al. (2016), i.e. D-allulose <
368 D-fructose < sucrose, does in fact match the order of Tg' values previously reported for the
369 three sugar solutions, i.e. D-allulose (-44 °C) < D-fructose (-42 °C) < sucrose (-32 °C) (Slade &
370 Levine, 1991).

371 3.2. Water Activity of Cupcakes

372 No significant interactions ($p<0.05$) were observed between sugar type and baking time,
373 when water activity values of the cupcakes were determined (Figure 2A and 2B). Cupcakes
374 formulated with D-allulose and D-fructose had significantly lower water activity values
375 ($p<0.05$) than did those formulated with sucrose, which mirrors the water activity findings
376 for these sugars in solution (Table 3). D-allulose and D-fructose have one more hydroxyl
377 group than sucrose, thus they can form more hydrogen bonds with water increasing their
378 water solubility compared to that of sucrose (Table 1). Typically, sugars with high solubility in
379 water and low molecular weight have the greatest effect on reducing water activity, through
380 their colligative effect in solution (Ergun, Lietha, & Hartel, 2010).

381 3.3 Weight Loss During Baking

382 Significant interactions were observed ($p<0.05$) between sugar type and baking time, when
383 weight loss during baking was studied (Figure 2C). The A12 and F12 cupcakes lost
384 significantly less weight ($p<0.05$), compared to the S12 cupcakes. As weight loss is a
385 consequence of water evaporation during baking (Dhen et al., 2016), these results indicate
386 that this process was occurring at a slower rate in D-allulose and D-fructose cupcakes, during
387 the initial stages of baking. It is possible that this was due to the greater water-holding ability
388 of these monosaccharides (Ergun et al., 2010). The water activity values for the D-allulose

and D-fructose solutions were significantly lower ($p<0.05$) than that for the sucrose solution (Table 3); consequently, there was less free water available in the D-allulose and D-fructose cupcake batters (Table 1), so more energy was required to initiate water evaporation during baking. Furthermore, based on colligative properties, due to their lower molecular weight, D-allulose and D-fructose solutions have higher boiling points than do sucrose solutions of equivalent weight percentage (Ergun et al., 2010). However, as baking time increased, the D-allulose cupcakes experienced an increase in the rate of weight loss, and consequently, the observed weight losses for the A20 and S20 cupcakes were not significantly different from each other ($p>0.05$). This might be because longer baking times gave place to higher temperatures in the crumb so a complete structural setting (starch gelatinisation and protein denaturation) and a higher rate of water evaporation, allowing further bubble expansion and water to be lost through open air cells. The resulting starch-filled protein network is so stiff that bubble expansion is constrained and air cells open, allowing steam to escape (van der Sman and Renzetti, 2020; Wilderjans et al. 2013). These results can be related to the pictures of S20 and A20 cupcakes in which cell opening and channelling can be observed (Figure 3)

D-fructose cupcakes, however, did not follow the same baking loss trend as the D-allulose cupcakes did, as baking time increased past 12 min. The F20 cupcakes experienced significantly more weight loss ($p<0.05$) than did both the S20 and A20 cupcakes. This finding differs from what has been reported in previous studies, in which cakes formulated with D-fructose did not differ significantly from those formulated with sucrose, both in terms of moisture loss during baking (Psimouli & Oreopoulou, 2012) and final moisture content (Kweon et al., 2016).

412 ### 3.4. Batter Viscosity

413 Sucrose, D-allulose and D-fructose batters showed shear thinning behaviour (data not
414 shown). In the range of shear rates studied, all batters showed similar apparent viscosity
415 values (data not shown). The experimental data showed good fit to the Ostwald deWaele
416 model (R^2 0.95-0.98); all batters showed similar consistency index (K) (data not shown).
417 Sucrose batters had a similar flow behaviour index (*n*) than D-allulose and D-fructose
418 batters; however, D-allulose batters showed a significantly higher *n* (values close to 1) than
419 D-fructose, indicating that the batter behaved closer to a Newtonian fluid. Initial batter
420 viscosity has an impact on the amount of air occluded and retained in the batter during
421 mixing, the stability of the air phase during early stages of mixing, on the evolution of bulk
422 viscosity during heating and thus, on the final quality of an aerated baked product (Martínez-
423 Cervera, Sanz, Salvador, & Fiszman, 2012). The results suggest that batters with the three
424 different sugars would have similar air phase occlusion and stability during the first steps of
425 the production process. However, very different cell expansion rate and structure setting
426 was observed (Figure 3) suggesting that the main effects of the different sugars in cupcake
427 structure formation were during the baking process.

428 ### 3.5. Cupcake Height and Crumb Cell Structure

429 There were significant interactions ($p<0.05$) between sugar type and baking time, when
430 cupcake height and cell crumb structure were studied (Figure 4A, 4B, and 4C). The sucrose
431 cupcakes were significantly taller ($p<0.05$) than the D-allulose and D-fructose cupcakes at all
432 baking times. Total air cell area for S12 was significantly higher ($p<0.05$) than for A12 and
433 F12 cupcakes. Moreover, the cell crumb structure of sucrose cupcakes did not experience
434 significant changes over increased baking times. The thermosetting of the batter, along with

435 gas expansion, bubble rupture and gas release, define the final cell crumb structure and
436 height of the cakes (Slade et al., 2021). In S20 samples there is an increase in the size of air
437 bubbles, due to CO_2 production, water evaporation and thermal expansion of the gases (50
438 °C) (van der Sman & Renzetti, 2020). Then the setting process of the batter starts; egg yolk
439 proteins denature, gluten thermosets, starch gelatinise, and egg white proteins denature
440 shortly after starch gelatinisation; in fact, it is a near-simultaneous occurrence of events
441 (Slade et al., 2021; van der Sman & Renzetti, 2020). Starch acts as a water-sink in cake and is
442 responsible for the transformation of a liquid foam into a solid foam (van der Sman &
443 Renzetti, 2020; Wilderjans, Luyts, Goesaert, Brijs, & Delcour, 2010); starch swells and leaches
444 amylose. During the thermal setting, a co-protein network is formed via S-S bridges between
445 denatured proteins from the wheat flour, egg yolk, and egg white (Lambrecht, Deleu,
446 Rombouts, & Delcour, 2018; Slade et al., 2021). The thermoset foam-structure is composed
447 by swollen starch granules entrapped in a protein matrix, with liquid oil (partially) coating
448 the swollen granules, and air bubbles encapsulated by denatured and aggregated protein
449 network (Slade et al., 2021; van der Sman & Renzetti, 2020). Upon further heating and
450 expansion, the bubble ruptures. Swelled starch granules, embedded in the protein network
451 as fillers, allow partial cell opening and provide resistance against normal forces avoiding
452 total collapse (Slade et al., 2021; van der Sman & Renzetti, 2020).

453 The smaller cell areas and height for the A12 and F12 cupcakes than that for the S12
454 cupcakes might be related to the effect of monosaccharides (D-allulose and D-fructose) in
455 shifting starch gelatinisation and protein denaturation temperatures as discussed in section
456 3.1. Samples with D-allulose and D-fructose showed lower wheat starch gelatinisation
457 temperatures than sucrose samples (Tables 3). In addition, it has been previously
458 demonstrated that low molecular weight plasticisers increase egg white protein

459 denaturation temperature by phase separation more than high molecular weight ones
460 (Renzetti et al., 2020). So, in D-allulose and D-fructose batters, starch gelatinisation started
461 earlier than in sucrose batters, initiating the thermal setting of the batter. In contrast, egg
462 white protein denaturation was delayed, increasing the separation of the onset
463 temperatures of the two main processes of the structure thermal setting: the starch
464 gelatinisation and the egg white denaturation. Figure 3 shows how the structure setting
465 process of the sucrose, D-allulose and D-fructose batters baked during different times. The
466 setting happened first at the bottom of the cupcakes, then at the centre and top, in
467 agreement with what has reported before (Wilderjans et al., 2013). In A12 and F12 batters,
468 the earlier start of starch gelatinisation, the asynchronous protein denaturation event and
469 the short baking time, did not allow the structure to fully expand and set; thus, the cupcakes
470 collapsed and shrunk showing very dense crumbs and low heights (Figure 3). Low molecular
471 weight plasticisers increase starch swelling and further solubilisation and leaching out of
472 amylose, than higher molecular weight plasticisers (Renzetti et al., 2020). Excessive starch
473 swelling with amylose leaching could hinder the formation of the protein network, as
474 previously observed with hydroxypropylated starches in cake systems (Wilderjans et al.,
475 2010). Moreover, starch gelatinisation increases batter viscosity and shear modulus due to
476 the swelling of the starch granules (van der Sman & Renzetti, 2020). This increase in viscosity
477 at earlier stages of baking could have limited the expansion of air cells. The impediments and
478 delay in the formation of the co-protein network that would strengthen the cell walls, and
479 the excessive swelling of the starch granules embedded in the network, gave place to a cake-
480 foam structure that was not able to withstand the increase in pressure in the expanding air
481 cells, thus cracks and channels could not be formed and the condensation of the water
482 vapour from the gas reduced the pressure and the cake collapsed. These cake-baking

phenomena has been previously described and explained in detail by Slade et al. (2021); van der Sman and Renzetti (2020); Wilderjans et al. (2013); Wilderjans et al. (2008).

The D-allulose and D-fructose cupcake heights increased significantly ($p<0.05$), as baking time was increased from 12 to 20 min (Figure 4A). Both the total air cell area and average cell size in the A20 and F20 cupcakes were significantly greater ($p<0.05$), compared to those for the A12 and F12 cupcakes, respectively (Figure 4B and 4C). The air cells in the A20 and F20 cupcakes were also of a significantly larger average size ($p<0.05$), compared to those in the S20 cupcakes. These results suggest that volume expansion was continuing in the D-allulose and D-fructose cupcakes, whereas sucrose cupcake expansion had terminated, after 12 min of baking. There could be two, complementary, explanations for this continuing expansion. Firstly, after 12 min of baking, the boiling point for water in the D-allulose and D-fructose cupcakes was achieved, thereby allowing the excess moisture to form water vapour, thus resulting in further air cell expansion.

Secondly, it could be possible that longer baking times allowed for a complete thermal setting process –involving wheat flour starch gelatinisation followed by egg protein denaturation– in the D-allulose and D-fructose cupcakes. The asynchrony of starch gelatinisation and protein denaturation will still be a limiting factor for the development of a homogeneous cell crumb structure. The formation of the co-protein network, although limited and at a later stage than the starch gelatinisation, would have allowed air cells to rupture, gases to escape and cell channels to form. A20 and F20 had experienced sufficient moisture evaporation, so that the cupcake crumb structure was likely to be in a rubbery (liquid) physical state, with a denatured egg protein-based network below its network Tg,

505 and thus supportive enough to be removed from the oven without collapse (Slade et al.,
506 2021) and resulting in more aerated cupcakes than at 12 min.

507 3.6. Cupcake Texture

508 Significant interactions were observed between sugar type and baking time, when textural
509 parameters were studied (Figure 4D, 4E, and 4F). The A12 and F12 cupcakes were
510 significantly harder, less cohesive, and less springy ($p<0.05$) than the S12 cupcakes were. The
511 greater hardness values for the A12 and F12 cupcakes are suggested to be attributable to
512 their denser crumb structure, due to limited expansion of their air phase, and structural
513 collapse, as described in section 3.4. It has been hypothesised that during the A12 and F12
514 thermal setting process, there was greater starch swelling and more amylose was leached
515 than in S12 cupcakes. During cupcake cooling, leached amylose will retrograde faster;
516 retrograded starch network is assumed to contribute to crumb firmness (van der Sman &
517 Renzetti, 2020), this could explain why A12 and F12 cupcakes were harder than the S20
518 cupcakes were. Cohesiveness and springiness are generally thought to indicate the
519 development of internal bonding in the protein network (Kalinga & Mishra, 2009; van der
520 Sman & Renzetti, 2020); thus, the lower cohesiveness and springiness of the A12 and F12
521 cupcakes are related to the limited and weaker co-protein network formation during the
522 thermal setting of these samples in comparison to S12.

523 The hardness of the D-allulose cupcakes decreased with increasing baking time, whilst their
524 springiness and cohesiveness increased, meaning that, after 20 min of baking, none of the
525 textural parameters of the D-allulose cupcakes were significantly different from those of the
526 sucrose cupcakes ($p>0.05$). This may have been due to further volume expansion, moisture
527 evaporation, and completion of thermal setting process.

3.7 Cupcake Colour

Significant interactions were observed ($p<0.05$) between sugar type and baking time, when cupcake colour and browning index were studied (Figure 5 and Figure 6A). Total colour differences were also calculated at each baking time (Table 4). The browning index and all colour parameters (L*a*b*) for the sucrose cupcake crumb and crust did not change significantly over baking time ($p>0.05$). In contrast, both the crust and crumb of the A20 and F20 cupcakes were significantly darker and redder ($p<0.05$) than the A12 and F12 cupcakes, and the browning index values for the A20 and F20 cupcake crusts were significantly higher than that for the S20 cupcake ($p<0.05$). At baking times of 16 and 20 min, the total colour differences between test cupcakes were appreciable by the human eye ($\Delta E^*>3$), when compared to sucrose control cupcakes. This indicates that browning was occurring more rapidly in the two ketohexose cupcakes, compared to in the sucrose cupcakes, which could be attributable to an increased rate of the Maillard reaction. D-allulose and D-fructose, unlike sucrose, are reducing sugars, so they can participate directly in the Maillard reaction with amino acids. Therefore, brown-coloured water-insoluble polymers known as melanoidins, which form during the final stage of the Maillard reaction, can be produced more readily (Starowicz & Zieliński, 2019). Enhanced rate of Maillard reaction, leading to the formation of brownish-coloured products, has also been widely reported for foods reformulated with D-allulose, including meringues (O'Charoen et al., 2014) and butter cookies (Sun et al., 2008). Caramelisation is another type of colour-forming reaction that can take place on the surface of cupcakes during baking (Purlis, 2010). It may be that this reaction also occurred more readily in the D-allulose and D-fructose cupcakes, because, as with the Maillard reaction, sucrose would first have to hydrolyse into its constituent

551 monosaccharides, fructose and glucose, before subsequent caramelisation reactions could
552 begin (Quintas, Brandão, & Silva, 2007).

553 3.8 Cupcake Acrylamide Concentration

554 In the context of the publication of studies speculating about a link between acrylamide and
555 cancer in humans (Besaratinia & Pfeifer, 2007), the acrylamide level in cupcakes is
556 considered to be an important factor to consider. Acrylamide forms during high-temperature
557 baking and other food processing methods above 120 °C in foods high in carbohydrates from
558 the reaction between free asparagine and intermediates of the Maillard reaction; in fact, it is
559 known that acrylamide creation during baking depends in part on the asparagine content of
560 the particular flour used (Chen, Wu, Fu, & Fan, 2020; Elmore, Koutsidis, Dodson, Mottram, &
561 Wedzicha, 2005). The free asparagine content in the UK wheat plain flour used, 0.27 (0.02)
562 (g/kg), was in the range of free asparagine values reported before in UK wheat flour
563 varieties, 0.21 (0.01) – 0.54 (0.02) (g/kg) (Hamlet, Sadd, & Liang, 2008).

564 Significant interactions were observed ($p<0.05$) between sugar type and baking time when
565 acrylamide concentration was studied (Figure 6B). There were no statistically significant
566 differences in acrylamide concentration among the A12, F12, and S12 cupcakes ($p>0.05$).
567 However, the A20 and F20 cupcakes had significantly higher ($p<0.05$) acrylamide
568 concentrations, compared to those in the A12 and F12 cupcakes, whereas the acrylamide
569 concentrations in the S20 and S12 cupcakes were not significantly different ($p>0.05$), which
570 suggests that, as baking time increased, the acrylamide concentration increased rapidly in
571 the D-allulose and D-fructose cupcakes, so that the A20 and F20 cupcakes ended up with
572 significantly higher ($p<0.05$) acrylamide concentrations than that in the S20 cupcakes. As
573 acrylamide is a product of the Maillard reaction, formed when reducing sugars (such as D-

574 allulose and D-fructose) react with the amino acid asparagine (Mottram, Wedzicha, &
575 Dodson, 2002), such as in wheat flour (Elmore et al., 2005) , these findings further indicate,
576 as would be expected, that the Maillard reaction was occurring more readily in the D-
577 allulose and D-fructose cupcakes than in the sucrose (a non-reducing sugar) cupcakes.
578 Moreover, when acrylamide concentration was plotted against crust browning index, a good
579 linear correlation ($R^2 = 0.75$) was observed (Figure 6C). At all three baking times, the
580 acrylamide concentrations in the D-allulose and D-fructose cupcakes were not significantly
581 different from each other ($p>0.05$), indicating that these two monosaccharides behaved
582 similarly, with regards to their extents of Maillard reaction. This, too, would be expected, as
583 they both have the same number of carbonyl groups available to react with the amine group
584 of free amino acids, which is the vital first stage in these reactions (Davis, 1995).

585 Although, as mentioned above, the acrylamide concentrations in the D-allulose and D-
586 fructose cupcakes were significantly higher than that in the sucrose cupcakes at 16 and 20
587 min of baking ($p<0.05$), these acrylamide levels are considered to be unlikely to be of
588 concern. Cupcakes are not a food currently covered by EU Regulation 2017/2158 (European-
589 Union, 2017) however, comparing results in the current study to benchmark levels for
590 similar products shows that the acrylamide levels in the D-allulose cupcakes are still minimal.
591 For example, the highest recorded acrylamide concentration in the D-allulose cupcakes in
592 this study was 15.1 µg/kg, which is just 4% of the benchmark level of 350 µg/kg for 'biscuits
593 and wafers'.

594 **4. Conclusion**

595 There were two major differences between the interactions that sucrose and D-allulose had
596 within the cupcake matrix. Firstly, D-allulose exhibited a greater water-holding ability than

that for sucrose; thus, cupcakes formulated with this monosaccharide experienced more limited water evaporation during early baking. Secondly, D-allulose did not retard starch gelatinisation as effectively as sucrose did during baking, as it has a lower effective numbers of hydroxyl groups available for intermolecular hydrogen bonding ($N_{OH,s}$) (Renzetti et al., 2021) than sucrose. However, as a monosaccharide, D-allulose, could have increased the protein denaturation time by phase separation (Renzetti et al., 2020) giving place to an asynchronous thermal setting of the cupcake batter. These changes in the main phase transitions during cupcake baking could have given place to an excessive starch swelling and amylose leaching and a limited formation of a co-protein network; thus, giving place to dense and compact cupcakes with higher hardness and lower cohesiveness and springiness than sucrose cupcakes. Additional studies would be needed to determine if the use of D-allulose could extend the shelf-life of cupcakes beyond that of traditional sucrose cupcakes and sensory experiments should be considered to determine sensory profile and acceptability of D-allulose cupcakes. Further research on cupcake reformulation strategies should be conducted to study combination of sugar replacers that create the conditions ($\Phi_{w,eff}$) and have the properties (molecular weight, $N_{OH,s}$) (Renzetti et al., 2021) to successfully replace sucrose in high moisture aerated systems like cupcakes Moreover, there are further opportunities for the use of this ultra-low-calorie sugar in other baked goods, which do not rely as heavily on water evaporation and volume expansion during processing for their final product quality. Biscuits, for example, which contain less water in their doughs and finished products, rise only slightly during the baking process, and experience limited starch gelatinisation during the baking process (Kweon et al., 2014), would be interesting to study further, using D-allulose as part of sucrose-reduced formulations, as previously reported by Young et al. (2016).

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