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Published Version

Gomez-Betancur, A. M., Lignou, S. ORCID: https://orcid.org/0000-0001-6971-2258, Pinarli, B., Norton, V. ORCID: https://orcid.org/0000-0002-1961-2539 and Rodriguez-Garcia, J. (2025) Effect of rare sugars on physical and sensory properties of doughs and biscuits. Food Hydrocolloids for Health, 8. 100230. ISSN 2667-0259 doi: 10.1016/j.fhfh.2025.100230 Available at https://centaur.reading.ac.uk/123846/

To link to this article DOI: http://dx.doi.org/10.1016/j.fhfh.2025.100230

Publisher: Elsevier

Food Hydrocolloids for Health 8 (2025) 100230

Contents lists available at ScienceDirect

Food Hydrocolloids for Health

journal homepage: www.elsevier.com/locate/fhfh

Effect of rare sugars on physical and sensory properties of doughs and biscuits

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ARTICLE INFO

Keywords:
Sucrose replacement
Allulose
Tagatose
Baked products
Physical properties

ABSTRACT

Replacing sucrose in baked products is challenging because of its unique contribution to texture and sweetness. Rare sugars like allulose and tagatose show potential as substitutes, displaying similar sweetness and acting as bulking agents. Although allulose and tagatose are epimers, it is unknown if they induce different behaviours on dough and biscuit characteristics. This study aimed to compare the impact of allulose and tagatose on thermal, rheological properties of dough, and on physical and sensory characteristics of biscuits. Four formulations were prepared using sucrose, fructose, allulose, and tagatose. Differential scanning calorimetry was performed on wheat flour-sugar solution mixtures, while rheological and texture analyses were performed on doughs. For biscuits, moisture content, water activity, dimensions, colour, texture, and sensory analysis were evaluated. Thermograms indicated that allulose and tagatose delayed the starch gelatinisation temperature (~82 °C) but to a lesser extent than sucrose (94.5 °C). Doughs with tagatose were approximately 54 % harder than those with sucrose, reflecting in higher complex modulus values during early-heating stages versus fructose, allulose or sucrose. The lower solubility of tagatose led to more system mobility and water interactions with wheat flour polymers, resulting in biscuits with lower spreading (7 mm), hardness (14.3 N) and increased moisture (8.4 %) compared to sucrose biscuits (8.7 mm, 16.9 N, 2.7 %, respectively). Biscuits made with allulose and fructose demonstrated intermediate moisture (~ 6 %) and diameter (~ 7.4 mm), a high browning index (~ 27.3), and texture similar to sucrose biscuits. These results suggest that despite having similar structures, allulose is a better sucrose replacer for biscuits than tagatose.

1. Introduction

Recent regulations such as the in-store promotion restrictions of products high in fat, sugar or salt (HFSS) in the UK (Food (Promotion and Placement) (England) Regulations, 2021) and front-of-pack nutrition warning labels in Canada, Australia, Latin America (Nutrition Labelling: Front-of-package nutrition symbol, 2024; UNICEF, 2021) have increased the need to reduce the levels of critical ingredients in processed foods, such as sugar. Particularly, in the UK, biscuits and baked goods are the main food category contributing to added-sugar intake for all age groups (British Nutrition Foundation, 2023).

Sucrose or table sugar is the most common sweetener used in the bakery industry and has a significant role in the making of biscuits (Slade et al., 2021; van der Sman & Renzetti, 2019). Sucrose competes with flour polymers for water, inhibiting undesired gluten development during mixing (van der Sman & Renzetti, 2019). At the stage of baking, it delays starch gelatinisation since the sugar solution acts as an anti-plasticiser, allowing a more pronounced dough spreading (Kweon et al., 2009). It also contributes to the final texture of the product as it recrystallises during cooling (Pareyt et al., 2009). In addition, sucrose participates in important colour and flavour-forming reactions and imparts sweet taste (Erdem et al., 2023; Garvey et al., 2021).

D-allulose (also known as d-psicose) and d-tagatose are rare sugars, ketohexoses, and epimers of fructose (C3 and C4, respectively) (O'Charoen et al., 2015) (see Fig. 1). In the last decade, these sugars have gained popularity as sucrose substitutes. For instance, allulose has 70 % sucrose sweetness, 0.4 kcal/g, negligible glycaemic index (GI) and has potential prebiotic effect (Zhang et al., 2023). Tagatose has a sweetness closer to sucrose (92 %), 1.5 kcal/g, a GI of 3, and exhibits prebiotic characteristics (Roy et al., 2018). In addition, these sugars can

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https://doi.org/10.1016/j.fhfh.2025.100230
Received 12 December 2024; Received in revised form 8 May 2025; Accepted 14 July 2025
Available online 16 July 2025
2667-0259/

be produced at large scale with enzymatic techniques from other sugars or carbohydrates (Roy et al., 2018; Zhang et al., 2023). Both rare sugars are generally considered as safe (GRAS) by the FDA. Although tagatose has been recently considered as added sugar by the FDA, it still has lower calorie intake and GI than polyols such as maltitol (2.4 kcal/g, GI:35) (Livesey, 2003). On the other hand, allulose is approved in many countries (e.g., South Korea, Japan, Australia and New Zealand, Mexico, USA) and holds a novel food status in the EU and the UK (A1247 Approval Report-Allulose-2024-AustraliaNZ, 2024; Southey, 2021). The latter highlights the importance of understanding the implications of using these ingredients in different food products.

Allulose and tagatose have been used to replace sucrose in cakes (Bolger et al., 2021; Hwang & Lee, 2018; Hwang et al., 2015; Lee et al., 2020a; Lee et al., 2020b; Özgür & Uçar, 2023) and biscuits (Jeong et al., 2024; Rojo-Poveda et al., 2020; Taylor et al., 2008; Woodbury & Mauer, 2024). Emphasising on studies performed on biscuits, most of them focused on the effect of these rare sugars on quality parameters such as colour, dimensions, breaking force (texture), and the evaluation of sensory hedonic perception. For instance, formulations with allulose (Jeong et al., 2024; Woodbury & Mauer, 2024) or tagatose (Rojo-Poveda et al., 2020; Taylor et al., 2008) produced biscuits with reduced lightness and increased redness, accounting to darker colour when compared to sucrose biscuits. In terms of dimensions, allulose and tagatose generated biscuits with lower width and more height in contrast to the sucrose counterpart (Jeong et al., 2024; Rojo-Poveda et al., 2020; Taylor et al., 2008; Woodbury & Mauer, 2024). Both studies assessing allulose as total replacer obtained that allulose biscuits had lower hardness than the control with sucrose (Jeong et al., 2024; Woodbury & Mauer, 2024). On the other hand, Taylor et al. (2008) found that tagatose biscuits were harder than sucrose biscuits, and Rojo-Poveda et al. (2020) did not find differences in the hardness between the two sugars. In terms of consumer studies, consumers preferred the sweetness of sucrose biscuits; however, in one of the studies, the appearance of biscuits with tagatose was preferred (Taylor et al., 2008), or showed no significant difference (allulose vs. sucrose) in colour preference as in the case of Jeong et al. (2024). In Taylor et al. (2008) study, the texture of tagatose and sucrose biscuits had similar liking. To the best of tour knowledge, there are no studies comparing the effect of allulose and tagatose in baked applications.

In addition, there has been an emerging interest in understanding the effect of allulose on the thermal and rheological properties of wheat flour-sugar solution mixtures (Bolger et al., 2021; Jeong et al., 2024; Woodbury & Mauer, 2024) and biscuit dough (Jeong et al., 2024; Woodbury & Mauer, 2024). These studies showed that allulose mixtures had lower gelatinisation temperature compared with sucrose which could explain the differences in biscuit size and texture (Bolger et al., 2021; Jeong et al., 2024; Woodbury & Mauer, 2024). Particularly, two of these studies also evaluated the effect of fructose, evidencing differences in the characteristics of biscuits and doughs (Jeong et al., 2024) and of cake and batter (Bolger et al., 2021) prepared with the two epimers (fructose and allulose); obtaining that fructose wheat-flour mixtures had slightly higher starch gelatinisation temperature than the allulose mixtures. This could partially explain the lower spreading of biscuits with allulose, in contrast to fructose in Jeong et al. (2024) work. The latter shows the relevance of individually assessing the performance of sucrose replacers, despite having similar molecular structure. However, it is important to mention that there is limited information on the behaviour of tagatose in the thermal-setting mechanism of biscuit dough; therefore, understanding this phenomena is necessary to be able to provide a scientific explanation of its effects on biscuit properties.

In agreement with previous findings between fructose and allulose, and acknowledging that tagatose is a third epimer, the hypothesis of this study is that tagatose has a different effect to allulose (and fructose) in the thermal-setting mechanism of biscuit dough, subsequently affecting the final product characteristics. Therefore, the aim of this study is to evaluate the effect of rare sugars on the thermal and rheological properties of biscuit doughs, and on the quality and sensory characteristics of sucrose-free biscuits. This research seeks to provide valuable insights to scientific and industry researchers by understanding the implications of using different rare sugars in low-moisture baked products, like biscuits.

2. Materials and methods

2.1. Ingredients and chemicals

The ingredients used to prepare biscuits were caster sucrose (Brakes, Kent, UK), d-tagatose (tagSweet®, Anderson Advanced Ingredients, Raalte, Netherlands), d-allulose (allSweet®, Anderson Advanced Ingredients, Raalte, Netherlands), fructose (Buywholefoodsonline.co.uk, Ramsgate, UK), non-fat dry milk (Brakes, Kent, UK), salt (NaCl), sodium bicarbonate (Hexeal Chemicals Ltd., Brundall, UK), refined palm oil (Fully HydroPalm, Cargill, Vilvoorde, Belgium), high-fructose corn syrup (HFCS) 42 % (ISOSWEET 111, Tereos, Aalst, Belgium), ammonium bicarbonate (Lovocado LTD, London, UK), plain wheat flour (moisture 13 %, protein 10.4 %, fat1.3 %; Mc Dougalls, Thame, South Oxfordshire, UK), and deionised water.

2.2. Thermal analysis of wheat flour and sugar solution mixtures

Differential Scanning Calorimetry (DSC) was performed to study the thermal properties (starch gelatinisation and protein denaturation) of wheat flour dispersed in sugar solutions. Particularly, starch gelatinisation temperature is associated with biscuits dough spread (Kweon et al., 2016). The DSC analysis was carried out following the method described by Kweon et al. (2009), with modifications. To prepare the mixtures, 50 % (w/w) sugar solutions of sucrose, fructose, allulose and tagatose were made in advance to avoid the formation of an endothermic peak during the heating process. For every mixture sample,

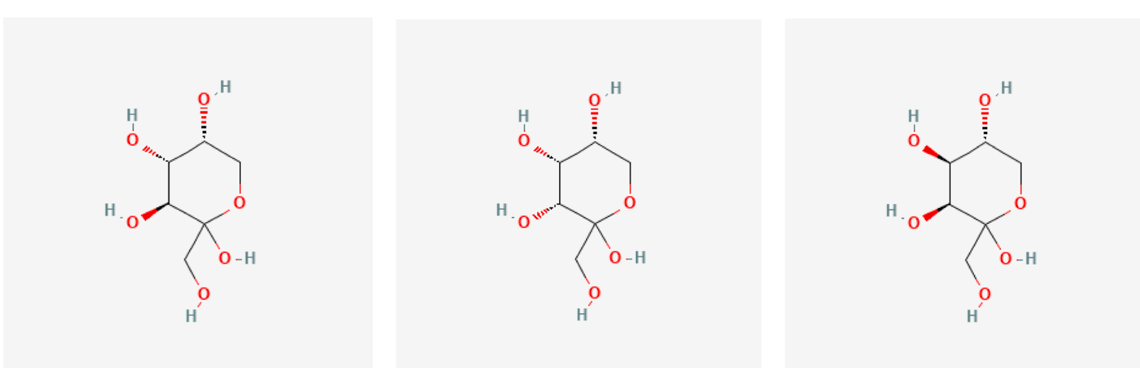

Fig. 1. 2-D ring chemical structures for **a)**d-fructose, **b)**d-allulose and **c)**d-tagatose (obtained from PubChem (nih.gov)).

wheat flour and sugar solution were mixed in 1:1 ratio by stirring with a spatula, until a homogeneous paste was obtained. A control sample without sugar was prepared by mixing water with wheat flour in 1:1 ratio. The mixture samples were stored in airtight containers and left at room temperature (21 °C) overnight. For every mixture, 20–25 mg were transferred to a T-zero aluminium DSC pan (TA Instruments, Wilmslow, UK) and were hermetically sealed. The sample pans and an empty pan as a reference were placed in the DSC instrument (TA-QA2000 DSC, TA Instruments, New Caste, Germany) and heated from 30 °C to 120 °C with a 10 °C/min heating rate. The equipment's calibration was completed before the analysis with indium and sapphire. The peak temperature and enthalpy were calculated by using TA Instruments Universal Analysis 2000 Software (version 4.5A Wilmslow, UK). The mixtures were prepared in triplicates and two analytical repeats for each mixture were performed.

2.3. Dough and biscuit preparation

Wire-cut biscuits were produced according to the American Association of Cereal Chemists method 10–53.01 (AACC, 1999). This official method was carried out in order to be able to compare the results of this study with previous works (Jeong et al., 2024; Kweon et al., 2009; van der Sman et al., 2022; Woodbury & Mauer, 2024). Four formulations, one with each sugar (sucrose, fructose, allulose, and tagatose) were prepared. The doughs were prepared in a free-standing electric mixer (Chef XL KV4100W, Kenwood, UK) by mixing 2.8 g of salt, 2.3 g of sodium bicarbonate, 94.5 g of sugar, 2.3 g of non-fat milk powder, and 90 g of shortening at speed 1 for 3 min, scraping every minute. Then, 1.1 g of pre-dissolved ammonium bicarbonate and 3.4 g of HFCS in 50 g of deionised water were added and mixed for 1 min at speed 1, and then for 1 min at speed 2. Next, 224.5 g of flour were added and mixed for 2 min at speed 1, scraping every 30 s. The obtained dough was carefully divided into eight oblong pieces (handling the dough as little as possible), placed in two previously greased baking sheets (four pieces per tray), rolled to 7 mm of height and cut with a cookie-cutter of 6 cm diameter. The eight dough pieces were baked in a deck oven (Polin Stratos, Verona, Italy) at 205 °C for 11 min (heating ratio: 70 % top and 30 % bottom). After baking, the biscuits were cooled down for 1 h before storing in sealed-propylene bags at room temperature (21 °C). The doughs and biscuits were prepared in triplicates (baking replicates). The doughs were analysed on the same day, and biscuits were analysed 24 h after.

2.4. Analyses performed on dough

2.4.1. Viscoelastic properties of dough

Most foods exhibit viscoelastic properties, including biscuit doughs. Understanding the changes in dough microstructure during heating provides valuable information on different phenomena such as fat melting, gluten development, and starch gelatinisation (Renoldi et al., 2021). The viscoelastic properties of doughs were studied using an oscillatory rheometer (MCR 302, Anton Paar Ltd., St Albans, UK) following the method described by Tsatsaragkou et al. (2021), using parallel serrated plates of 25 mm diameter (profile 1 mm x 0.5 mm). After loading the sample, the gap between the plates was set at 1.8 mm, the edges of excess dough were carefully removed with a spatula and covered with silicon oil to avoid evaporation. Then, the sample was allowed to rest for 10 min to ensure relaxation and temperature equilibration for the measurement at 35 °C (dough temperature when moulding and cutting at industrial scale). An amplitude sweep test was performed to identify the linear viscoelastic (LVE) region. The shear storage modulus (G') and the loss modulus (G'') values, which define elastic and viscous behaviour of samples, respectively, were recorded.

A temperature sweep test was carried out from 35 °C to 140 °C with 5 °C/min heating rate, at 0.04 % strain (in the viscoelastic linear regime). The shear storage modulus (G'), the loss modulus (G''), and the complex modulus (G^*) were recorded. The complex modulus (G^*) value was calculated using Eq. (1):

$$G^* = \sqrt{(G')^2 + (G'')^2} \tag{1}$$

2.4.2. Texture properties of dough

A dough consistency test was performed with a Texture Analyser (TA-XT2, Plus-Upgrade, Stable Micro Systems, Godalming, UK), using a 5 mm diameter spherical stainless-steel probe, and a 5 kg load cell. Dough pieces (6 cm diameter and 7 mm height) were placed on the centre of the platform and compressed at 60 % of their height with a test speed of 1 mm/s. The maximum force (N) values were recorded by the Exponent software (version 6.1.18.0, Stable Micro Systems, Surrey, UK). For each dough batch, eight dough pieces were analysed.

2.5. Quality characteristics of biscuits

2.5.1. Moisture content and water activity

Eight biscuits from each formulation batch were ground for 1 min in a food grinder (CH180, Kenwood, UK). Moisture content of the biscuits was determined with a moisture analyser (MA 150, Sartorius, Germany), using approximately 3 g of biscuit crumbs and drying them at 130 °C until constant weight. Water activity of the biscuits was determined with a HygroLab C1 (Rotronic AG, Basserdorf, Switzerland). These analyses were performed in duplicates, per each baking replicate.

2.5.2. Biscuit dimensions

Biscuits dimensions were measured according to the AACC method 10–53.01 (AACC, 1999) using a calliper. Four biscuits from the same tray were placed edge to edge to measure the width. Then, the biscuits were rotated 90°, and the length was measured. Biscuit height was measured by stacking four biscuits on top of each other and reading to the nearest ½ mm at the centre of the top biscuit; then, the four biscuits were restacked by changing the order and orientation of the biscuits. The obtained values from each measurement for width, length, and height were divided by four to obtain the mean values, per baking replicate. Two sets of four biscuits were used per baking replicate.

2.5.3. Colour measurements

Biscuit colour was analysed with a chroma meter (CR-400, Konica Minolta, Tokyo, Japan). The measurements were taken at the top-centre of the biscuits and results were expressed in accordance with the CIELAB system (illuminant C and 10° viewing angle). The coordinates measured were L^* ($L^* = 0$ [black], $L^* = 100$ [white], a^* ($+a^* =$ redness and $-a^* =$ greenness) and b^* ($+b^* =$ yellowness and $-b^* =$ blueness) (Bodart et al., 2008). The browning index (*BI*) and colour difference (ΔE^*) were calculated using the Eqs. (2), (3), and (4) (Francis & Clydesdale, 1976; López-Malo et al., 1998). For each baking replicate, eight analytical repeats were performed.

$$BI = \frac{(x - 0.31)}{0.172} \times 100 \tag{2}$$

Where x is obtained from:

$$x = \frac{(a^* + 1.75L^*)}{5.645L^* + a^* - 0.3012b^*} \tag{3}$$

$$\Delta E^* = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \tag{4}$$

Where L_0^*, a_0^* and b_0^* are the coordinates from the sucrose biscuits, used as the reference sample. In addition, to determine whether the colour difference was visually obvious, the following values of overall colour difference were used as reference: $\Delta E^* < 1$, colour differences are not obvious for the human eye; $1 < \Delta E^* < 3$, minor colour differences could be appreciated by the human eye depending on the hue, and Chroma; $\Delta E^* > 3$ colour differences are obvious for the human eye

(Bodart et al., 2008).

2.5.4. Texture properties of biscuits

A fracture test was performed on biscuits with the same instrument from *2.4.2.,* using a three-point bending probe (A/3 PB) and a 5 kg load cell. The experimental conditions were test-speed: 1 mm/s, distance between supports 4.5 mm, and trigger force 0.2 N. The force at break (N) and the distance at break (mm) were measured and analysed as fracture strength and fracturability, respectively. For each baking replicate, eight biscuits were analysed.

2.6. Sensory evaluation of the biscuits

The sensory profile of the biscuits with different sugars was carried out using descriptive sensory profiling (International Organisation for Standardization, 2021, 2023; Stone & Sidel, 2004). The trained sensory panel at the Sensory Science Centre (University of Reading, $n = 14$; 13 female and 1 male) with at least two years of experience was used. The panel developed a consensus vocabulary of the sensory attributes across different modalities (appearance, odour/aroma, taste/flavour, mouthfeel and aftereffects) using reference standards and descriptions to assist defining attributes. In the supplementary Table S1 a definition of the evaluated attributes along with their anchors is presented. For scoring sweet taste, a structured scale with five reference anchors was employed. The reference anchors were five sucrose solutions of increasing sweetness (2, 3, 4, 5 and 6 % w/v: anchor point 1 [low] = 0, 2 = 25, 3 = 50, 4 = 75, 5 [strong] = 100). The samples (half biscuit; 7 mm height x 3 cm diameter) were presented to the panellists at each session on a 3-digit coded ceramic grey plate. Scoring sessions were carried out in duplicate and biscuits were presented in a balanced monadic sequential design. The attributes were scored on visual analogue scales (VAS) (0–100) with anchored extremes using Compusense Cloud Software (Compusense Inc., Guelph, ON, Canada). Panellists were previously familiarised with the sucrose solutions during training sessions. There was a 30 s pause after the end of the mouthfeel attributes; then, the assessors scored the after-effects. In order to minimise carryover effects, a 2 min interval was allowed between each sample. Panellists were asked to cleanse their palate between tastings with filtered water. All the assessments were carried out in isolated booths under artificial daylight and at room temperature (22 °C).

2.7. Statistical analysis

The quantitative data for each measurement were analysed by one-way analysis of variance (ANOVA) using XLSTAT software package (version 2023, Addinsoft, Paris, France). For those results exhibiting significant difference, Fisher's least significant difference (LSD) post-hoc test was applied to determine which sample means differed significantly ($p < 0.05$). SENPAQ (version 5.01; Qi Statistics, West Malling, UK) was used to carry out two-way ANOVA with sample fitted as fixed effect, and panellists as random effect. Both main effects were tested against the sample by panellist interaction and Fisher's LSD test was used for the multiple comparisons between samples.

3. Results and discussion

3.1. Thermal properties of wheat flour and sugar solution mixtures

The thermograms for the wheat flour dispersion and the wheat flour-sugar solution mixtures are shown in Fig. 2. A characteristic endothermic peak corresponding to starch gelatinisation (T_{peak} or T_g) is observed in all samples; highlighting that all the sugars significantly delayed the starch gelatinisation temperature compared to the wheat flour dispersion ($p < 0.05$). Sucrose dispersion had the highest T_g followed by fructose, and allulose and tagatose. One hypothesis explaining the shift of T_g in wheat flour mixtures with sugars is based on the molecular weight (MW) of the water-sugar solutions; when water is used by itself in a wheat flour dispersion it acts as a plasticiser and lowers the T_g of the amorphous material which in this case were starch and gluten polymer (Slade et al., 2021). However, when sugars are added, the solution's weight-average MW increases causing the contrary effect (antiplasticising), thus raising the T_g (Slade et al. 2021). This hypothesis confirms why the three epimers (MW: 180 g/mol) had lower T_g and the stronger antiplasticiser effect of the sucrose dispersion (MW: 342 g/mol). In addition to the MW, the volumetric density of hydrogen bonds in solution ($N_{OH,s}$) complements the explanation for the observed

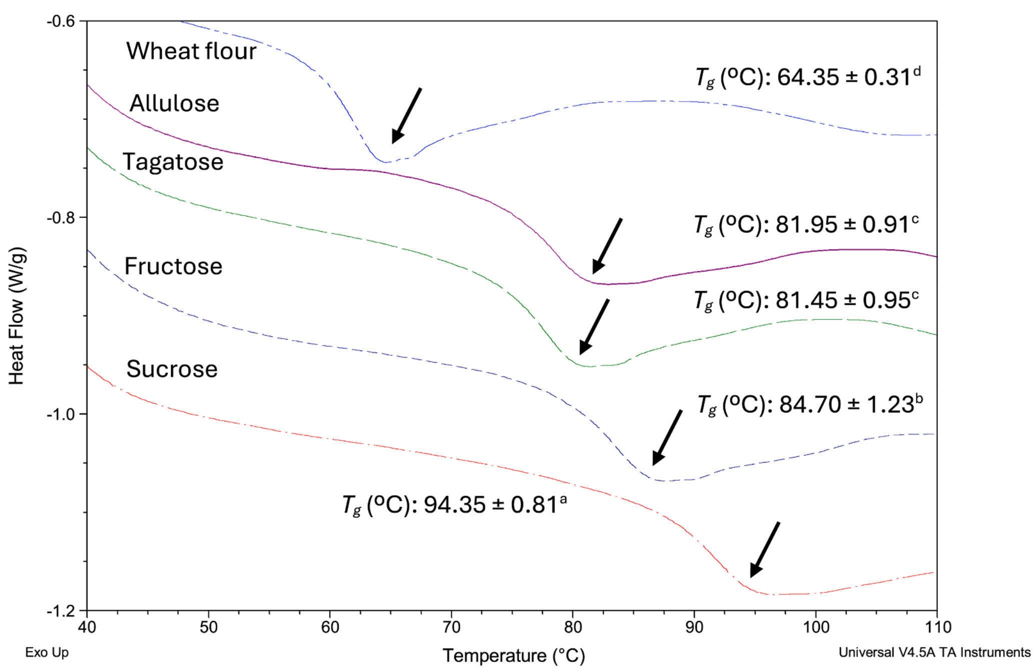

Fig. 2. Thermograms for wheat flour dispersion and wheat flour-sugar solution mixtures with values for T_g. The arrows are pointing at the T_g. Means with different letters were significantly different (p-value < 0.05).

differences in T_g. Sucrose has higher $N_{OH,s}$ (4.48), followed by fructose (3.98), tagatose (3.59), and allulose (3.35), meaning that sucrose can form more intermolecular bonds with water; making it less available to interact with starch (van der Sman & Mauer, 2019; Woodbury & Mauer, 2022).

$N_{OH,s}$ values could also explain why fructose had a slightly higher antiplasticising effect in contrast to allulose. These results were in line with previous results from Bolger et al. (2021) and Jeong et al. (2024). In addition, these results present for the first time that allulose and tagatose have similar T_g. In this matter, although these two sugars have different $N_{OH,s}$ values, this property alone does not fully explain their similar starch gelatinisation behaviour. A potential explanation relies on the stereochemistry and mutarotation of the sugars in solution (Tas et al., 2022). The reorganisation (mutarotation) and stereochemistry of the molecules when dissolved in water has been studied by Köpper and Freimund (2003). Through NMR analyses they evidenced that in solution, d-fructose is mainly in a β-pyranose form (72 %), whereas tagatose is more likely to be in a α-pyranose form (79 %), and different anomers coexist in solution for d-psicose or d-allulose (α-pyranose ∼ 22 %, β-pyranose ∼ 24 %, and α-furanose ∼ 39 %, β-furanose ∼ 15 %, respectively). According to the literature, the β-anomers have higher number of water oxygens readily available to react with water compared with the α-anomers, which could explain the increased T_g value in fructose in contrast to allulose and tagatose, who have more abundance of alpha anomers (∼61 and 79 %, respectively) (Tas et al., 2022).

3.2. Rheological properties of biscuit doughs

Changes in the microstructure of the doughs during heating are represented in Fig. 3. The behaviour of the complex shear modulus (G^*) during temperature sweeps could be described in three segments (zone I, zone II, and zone III). **Zone I** shows a drop in G^* values in all the samples. In the rare sugars and fructose doughs, this drop occurred from 35 to 55 °C, whereas for sucrose dough, it continued until 65 °C. This decrease in G^* could be explained by the melting of fat, dissolution of sugars and the higher kinetic energy of molecules that reduces the binding interactions among them (Laguna et al., 2013). In this temperature range, G^* values were visually different among all the dough samples. In this context, tagatose dough exhibited the highest G^* values followed by sucrose, fructose, and allulose doughs. These differences could indicate a higher structuration on the tagatose dough, which could be linked to the reduced solubility of tagatose in water (58 % at 21 °C) (Khuwijitjaru et al., 2018) in contrast to the higher solubility of the other sugars (sucrose, 67 %; fructose, 80 %; allulose, 74 % at 25 °C) (Bolger et al., 2021), which follows the same order of G^* values in this region. This suggests that solubility of sugars in water is the principal variable conditioning the viscoelasticity of doughs at the earlier stages of baking.

In **zone II**, the temperature sweep showed a plateau in the behaviour of G^* for fructose and rare sugar doughs, indicating no major microstructural changes were taking place within this range of temperatures (55 to 80 °C). In contrast, the sucrose dough exhibited a steady decline in G^* values until 86 °C. In this zone, tagatose continued having the highest G^* values, followed by fructose and allulose, and finally, sucrose. A reason for the weaker viscoelastic behaviour of sucrose could indicate that within this temperature range, the sucrose dispersion present in the dough continues decreasing in viscosity and that starch granules embedded less water, as evidenced by Jeong et al. (2024) in microstructural analysis of sugar-wheat flour dispersions at 50 °C and 80 °C, where they observed that starch granules at 80 °C were swollen in the allulose and fructose samples, whereas starch granules remained intact in the sucrose sample. The previous also partially explain the higher viscosity in the fructose, allulose, and tagatose doughs within this temperature range. To support this affirmation, the T_{onset} obtained from the DSC graphs showed that starch gelatinisation in the sucrose samples started around 86 °C while T_{onset} for allulose, tagatose, and fructose was in a range of 73–77 °C (see Table S2) which is consistent with the present viscoelastic results.

Lastly, **zone III** exhibited a rise in the value of G^* for all the samples. It is important to note that this increase started earlier (around 81–84 °C) for fructose, allulose, and tagatose doughs than for sucrose dough (around 93 °C). These results are consistent with the obtained T_g values (*Section 3.1.*), confirming that sucrose was a more efficient antiplasticiser than the three epimers. In this region, until 120 °C, tagatose

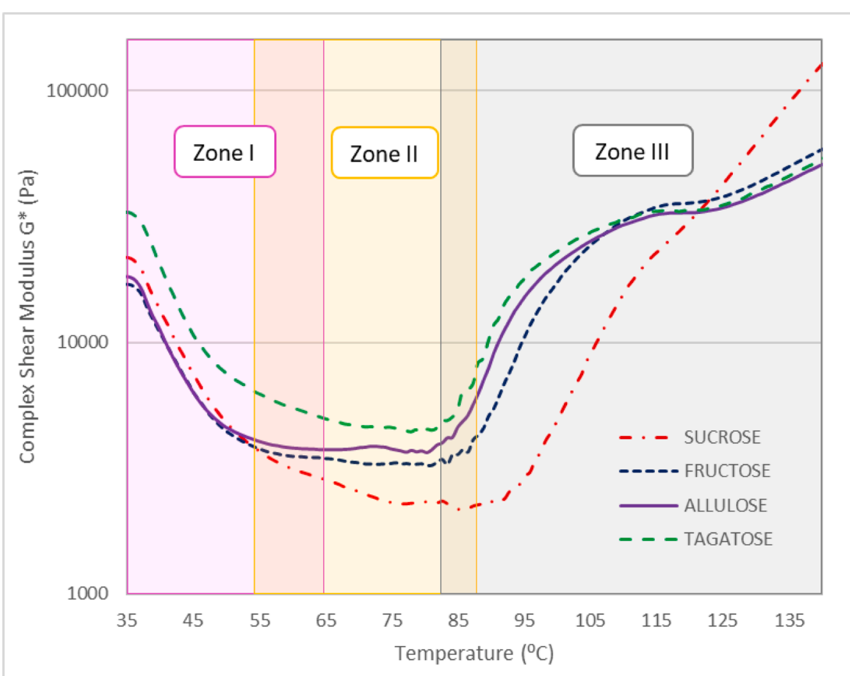

Fig. 3. Complex modulus (G^*) in function of temperature in biscuit doughs, where zone I (pink grid), zone II (yellow grid) and zone III (grey grid) delimit early, intermediate and late stages of heating, respectively.

dough continued having higher values of G^*, followed by allulose = fructose > sucrose doughs; however, from 120 °C until the end of the analysis, the sucrose dough had the highest values of G^*. This increase in solid-like structuring of sucrose dough at higher temperatures could denote that sucrose molecule is undergoing hydrolysis and could also be exhibiting a phase change (from crystalline to amorphous) (Renzetti & Jurgens, 2016).

Even though some authors have evaluated the effect of sucrose, fructose, and allulose on the rheological characteristics of wheat flour and sugar mixtures (Jeong et al., 2024; Woodbury & Mauer, 2023), the current approach using actual biscuit dough accounts for a more realistic behaviour of the viscoelasticity of this system during baking.

3.3. Dough textural properties

Results from the dough compression test are shown in Fig. 4. Doughs prepared with tagatose exhibited the greatest hardness ($p < 0.05$), followed by sucrose, and then fructose and allulose doughs. These results agree with the rheological properties of the doughs at the early stage of heating (Fig. 3, **zone I**, 35 – 55 °C). Similar results were reported by Taylor et al. (2008) when investigating the effect of tagatose as a sucrose replacer in sugar-type cookies and by Woodbury and Mauer (2024) when studying the impacts of allulose in wire-cut biscuit properties. The higher softness of fructose and allulose doughs could be explained by the higher solubility of these sugars compared with tagatose, as discussed in *Section 3.2*. Higher sugar solubility increases the total solvent amount in the dough, giving place to softer doughs (Kweon et al., 2009). In addition, when the concentration of the sugar solution, as a solvent, increases, the kinetics of gluten development are hindered, giving place to softer doughs (Kweon et al. 2009). The latter could indicate that the higher G^* of tagatose dough noticed in Fig. 3 (zone I) and increased hardness (Fig. 4) could result from more water interacting with gluten proteins.

It is important to note that despite allulose, tagatose, and fructose being epimers, the current results confirm the hypothesis that even similar molecular structures could exhibit different behaviour in doughs' physical properties. In this sense, although the mutarotation of the sugars had less impact on the thermal properties of the evaluated wheat flour and sugar mixtures, it seems relevant in determining the rheological behaviour and texture of the biscuit doughs.

3.4. Moisture and water activity of biscuits

The results for moisture, water activity (a_w), dimensions, texture, and colour of the biscuits elaborated with different sugars are presented in Table 1. Significant differences were observed for the stability variables, where tagatose biscuits presented the highest moisture content and a_w, followed by allulose, fructose, and sucrose samples ($p < 0.05$). These results agreed with those from Woodbury and Mauer (2024), who also obtained higher moisture content in allulose biscuits than in biscuits with sucrose. The lower solubility of tagatose could have caused more water-binding to starch and proteins, confirmed by the increased a_w. This theory could also explain why the fructose and allulose biscuits exhibited lower values of moisture and a_w than tagatose ones, because although they presented similar T_g as tagatose, they had higher solubility values and lower values of G^*, meaning less water took part in the formation of gelatinised starch and gluten network, and more water was available for evaporation. Lastly, the even lower values of moisture and a_w in the sucrose samples could be explained by the reduced formation and interaction of water holding networks, such as gelatinised starch and gluten, as this sample presented the highest T_g and the lowest G^* until 120 °C.

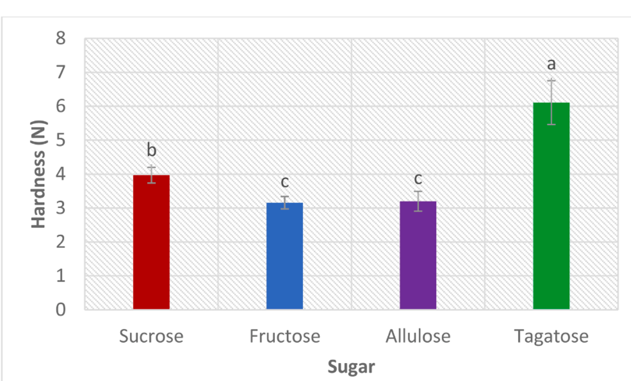

Fig. 4. Hardness of biscuit doughs. Bars with different letters were significantly different (p-value < 0.05).

3.5. Biscuit dimensions

Results for the biscuits dimensions are presented in Table 1 and a representative image of the biscuits is portrayed in Fig. 5. Sucrose biscuits exhibited significantly greater ($p < 0.05$) spreading (higher width and length, and less height), followed by those with fructose, allulose, and tagatose. Rheological results of the biscuit dough supported these results, as low values of G^*, especially in the stages before starch gelatinisation (Fig. 3, zone II), gave place to a greater spreading of the sucrose samples before it settled into a solid-like product. A similar order of spreading was observed by Jeong et al. (2024) between sucrose, fructose, and allulose biscuits.

3.6. Texture of biscuits

In terms of texture, samples with sucrose, fructose, and allulose exhibited a similar fracture strength ($p > 0.05$). However, tagatose had the lowest fracture strength ($p < 0.05$) (Table 1). On the other hand, tagatose had significantly higher fracturability ($p < 0.05$) followed by fructose and allulose, and lastly, sucrose samples. These results indicated that sucrose biscuits were more fragile (less resistant to break), whereas, in tagatose biscuits the probe needed to cross more distance before breaking the biscuit. The lower hardness of tagatose biscuits could be related with the higher moisture levels on this sample, linked to the increased interactions of water with gluten proteins and gelatinised starch (Kweon et al., 2009). This could explain the higher values of fracturability in tagatose biscuits as a slower crack propagation took place, coming from plastic deformation from the water holding networks (Laguna et al., 2013; Woodbury & Mauer, 2024). In another work, authors obtained harder biscuits with total replacement with tagatose and much lower hardness with fructose; nevertheless, they used a different recipe and different baking times among the samples (Taylor et al., 2008). This raises the importance of using standard recipes and baking methods to allow consistent comparisons between investigations. For instance, Kweon et al. (2009) compared two official methods (sugar-snap and wire-cut) to investigate the functionality of sugars in biscuit making, obtaining better results with the wire-cut recipe, which is the one used in the present study.

The lower fracturability of sucrose sample is consistent with other works (Laguna et al., 2013; Rodriguez-Garcia et al., 2022; Woodbury & Mauer, 2024); however, the trend in fracture strength was different from the results obtained by Jeong et al. (2024) and Woodbury and Mauer (2024), since their biscuits with sucrose required higher snapping force than allulose ones.

3.7. Colour of biscuits

The colour was significantly different ($p < 0,05$) among all the biscuit samples in all the coordinates (L^*, a^*, and b^*) and browning index (*BI*) (see Table 1). Sucrose biscuits were the lightest and presented the least redness, yellowness and browning values ($p < 0.05$) as explained

Table 1
Quality characteristics of biscuits elaborated with different sugars.

Measurement		Sucrose	Fructose	Allulose	Tagatose	Significance of the sample (p-value)
Stability	Moisture (%)	2.68 ± 0.34^{a}	5.59 ± 0.41^{b}	6.47 ± 0.17^{c}	8.39 ± 0.41^{d}	<0.0001
	aw	0.26 ± 0.01^{a}	0.45 ± 0.01^{b}	0.50 ± 0.02^{c}	0.59 ± 0.01^{d}	<0.0001
Dimensions	Width (cm)	8.54 ± 0.08^{a}	7.56 ± 0.04^{b}	7.15 ± 0.03^{c}	7.07 ± 0.02^{d}	<0.0001
	Length (cm)	8.85 ± 0.11^{a}	7.89 ± 0.08^{b}	7.13 ± 0.06^{c}	6.99 ± 0.06^{d}	<0.0001
	Height (cm)	0.87 ± 0.03^{d}	1.16 ± 0.04^{c}	1.23 ± 0.02^{b}	1.42 ± 0.03^{a}	<0.0001
Texture	Fracture strength (N)	16.87 ± 2.43^{a}	17.08 ± 1.52^{a}	17.49 ± 1.75^{a}	14.03 ± 0.96^{b}	<0.0001
	Fracturability (mm)	35.1 ± 0.33^{c}	37.25 ± 0.26^{b}	37.18 ± 0.29^{b}	38.27 ± 0.34^{a}	<0.0001
Colour	L^*	62.11 ± 0.64^{a}	49.23 ± 1.05^{d}	50.94 ± 1.50^{c}	55.54 ± 1.5^{b}	<0.0001
	a^*	8.47 ± 0.41^{c}	14.82 ± 0.33^{a}	14.63 ± 0.44^{a}	12.85 ± 0.66^{b}	<0.0001
	b^*	34.39 ± 0.35^{d}	35.17 ± 0.57^{c}	35.59 ± 0.89^{b}	37.20 ± 0.52^{a}	<0.0001
	BI	15.11 ± 0.62^{d}	27.86 ± 0.88^{a}	26.80 ± 1.16^{b}	22.81 ± 1.35^{c}	<0.0001
	ΔE^*	0	14.38	12.81	8.38	

Means in the same row without a common letter are significantly different ($p < 0.05$) according to Fisher's LSD test.

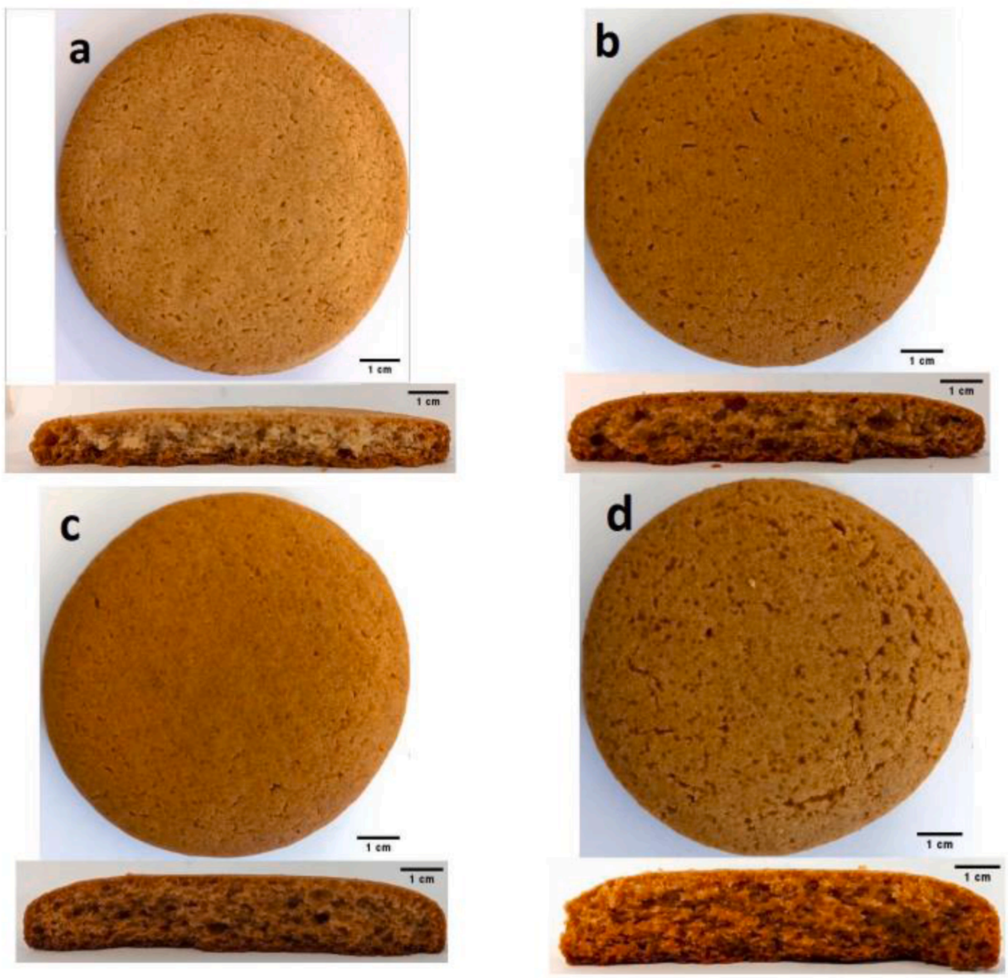

Fig. 5. Surface and cross-section pictures of biscuits prepared with a) sucrose, b) fructose, c) allulose, and d) tagatose.

by the parameters L^*, a^*, b^*, and *BI*, respectively. Fructose and allulose biscuits were significantly darker and presented higher redness values ($+ a^*$) and the highest *BI* among all samples. Interestingly, biscuits elaborated with tagatose were slightly lighter, had a lower *BI* than those elaborated with fructose and allulose and showed the highest yellowness ($+b^*$) among all samples. In addition, the overall colour difference showed that all the samples with sucrose replacers were significantly different in colour from the reference, the sucrose biscuit ($\Delta E^* > 3$) (Bodart et al., 2008), as seen in Fig. 5.

The increased browning on formulations with reducing sugars (e.g., fructose, allulose, and tagatose) was expected since these molecules are more readily available to participate in Maillard Reaction and caramelisation in contrast to sucrose, which needs hydrolysation before participating in these reactions (Quintas et al., 2007). Melanoidins are pigments produced at later stages of the browning reactions meaning that samples with rare sugars and fructose could probably present higher concentration of melanoidins, and perhaps, more concentration of other compounds derived from browning reactions, such as volatile organic compounds (VOCs) and process contaminants like acrylamide (Göncüoğlu Taş et al., 2023; Helou et al., 2016). The current results agreed with other authors who also evaluated biscuits colour with sucrose and allulose (Woodbury & Mauer, 2024) and with sucrose, fructose, and allulose (Jeong et al., 2024) and with results from aqueous model systems with β-lactoglobulin, where d-psicose (allulose) showed more browning (absorbance at 420 nm) than d-fructose (Zeng et al., 2013).

Nevertheless, in another aqueous model system with glycine, d-tagatose produced more browning, followed by d-allulose and d-fructose (Baek et al., 2008), which is contradictory with the present results. In this regard, it is important to mention that the increased moisture content of the tagatose sample could have delayed the kinetics of the browning reactions, since compounds like acrylamide, which are highly linked to increased colour in the surface of foods are favoured in low-moisture systems (Parker et al., 2012; Zhang et al., 2007).

3.8. Sensory evaluation of biscuits

The trained panel generated a vocabulary consisting of 30 attributes. Table 2 gives the mean panel scores for the attributes and the significant differences for the samples as determined by ANOVA. From this table, 21 attributes out of 30 were found to be significantly different between the four samples. According to the panellists, the biscuits with rare sugars and fructose showed increased golden colour than those with sucrose. The order of golden colour agrees with the *BI* (fructose > allulose > tagatose > sucrose) (see Table 1), which can also be observed in Fig. 5. Moreover, it confirms that the assessors identified the colour differences ($\Delta E^* > 3$) since the samples with tagatose were perceived as lighter in contrast to the fructose and allulose ones. The biscuits with tagatose showed an uneven top surface (presence of cracks and dark spots, as noted in Fig. 5) that could come from undissolved sugar, explained by its low solubility. In contrast, sucrose, fructose, and allulose had a more even surface. Even though the instrumental analyses showed differences in the dimensions of the biscuits (see Table 1 and Fig. 5), this seemed not to affect the overall scoring of the density of crumb, though the samples with monosaccharides presented a slightly higher density of crumb.

The results from the aroma and flavour showed that there are significant differences in most of these attributes ($p < 0.05$), except for the salty and vegetable oil (fatty) flavour. All the after-effects linked to odour/taste exhibited significant differences. Attributes such as sweet (aroma, flavour, after-effect), floury (aroma and flavour), and popcorn aroma were significantly higher for the sucrose biscuits, whereas attributes such as baked aroma, brown spices aroma, golden syrup (aroma and flavour), bitter (flavour and after-effect), and burnt (flavour and after-effect) were significantly higher in the fructose and rare sugar samples. The increased intensity of these aromas and flavours in the samples with fructose, allulose, and tagatose could be derived from the pronounced browning reactions (e.g., Maillard, caramelisation) as explained earlier, which are responsible not only for the colour, but also for the generation of aroma volatile compounds (Parker, 2015a, 2015b). Though previous authors had also reported higher sweetness in sucrose biscuits versus fructose and allulose biscuits (Jeong et al., 2024), and consumer evaluations showed a preference for the sucrose biscuits sweetness in contrast to tagatose biscuits (Taylor et al., 2008), it is important to note this is the first study performing a more comprehensive assessment of the sensory characteristics of biscuits made with rare

Table 2
Mean panel scores for sensory attributes of biscuits elaborated with different sugars.

Attributes	Scores[a]				LSD[b]	Significance of the sample (p-value)[c]
	Sucrose	Fructose	Allulose	Tagatose		
Appearance						
Golden colour (top surface)	34.1[d]	70.73[a]	62.00[b]	56.43[c]	7.5	**< 0.0001**
Uneven top surface (cracks)	37.45[b]	29[b,c]	23.67[c]	53.85[a]	8.3	**< 0.0001**
Density of crumb	44.93	52.95	50.51	53.24	12.5	0.5087
Aroma						
Baked	36.58[b]	59.55[a]	58.27[a]	52.93[a]	7.8	**< 0.0001**
Sweet	38.94[a]	26.92[b]	29.58[b]	29.69[b]	7.4	**0.0124**
Brown spices	0.06[b]	20.49[a]	22.5[a]	26.78[a]	7.6	**< 0.0001**
Golden syrup	1.28[c]	12.6[b]	22.02[a]	19.44[a]	6.3	**< 0.0001**
Floury	24.03[a]	6.83[b]	5.11[b]	4.6[b]	7.7	**< 0.0001**
Popcorn	34.97[a]	1.77[b]	0.24[b]	0.61[b]	7.8	**< 0.0001**
Taste/Flavour						
Sweet	67.24[a]	35.74b	20.02[c]	25.78[c]	7.3	**< 0.0001**
Salty	7.37	10.36	9.07	8.95	3.3	0.3595
Bitter	0.42[c]	26.58[a,b]	30.15[a]	21.21[b]	7.1	**< 0.0001**
Floury	24.05[a]	9.78[b]	10.49[b]	13.36[b]	7.3	**0.0018**
Vegatable oil (fatty)	3.49	4.93	4.68	5.12	3.7	0.8115
Golden syrup	4.49[b]	11.19[a]	13.75[a]	13.6[a]	5.5	**0.0097**
Burnt	0.65[c]	38.09[a]	34.92[a]	23.86[b]	9.1	**< 0.0001**
Texture/mouthfeel						
Hardness of first bite	64.62[a]	49.75[b]	36.02[c]	24.38[d]	7.2	**< 0.0001**
Crunchy	63.43[a]	37.9[b]	20.97[c]	9.39[d]	7.7	**< 0.0001**
Drying	31.47	30.46	33.76	31.95	6.3	0.6171
Mouth coating	27.37[b]	31.69[a,b]	34.31[a]	34.85[a]	5.5	**0.0301**
Pasty	22.73[b]	31.25[a]	34.9[a]	36.26[a]	6.9	**0.0018**
Body	27.13[c]	45.53[b]	54.88[a]	57.35[a]	7.8	**< 0.0001**
Tooth packing	32.83	30.74	34	34.08	6.8	0.6586
After effects						
Sweet	44.16[a]	25.98[b]	16.93[c]	22.75[b]	5.2	**< 0.0001**
Bitter	1.66[c]	21.97[a,b]	25.49[a]	16.01[b]	6.3	**< 0.0001**
Salivating	31.98	27.64	29.4	29.61	4.2	0.1473
Drying	28.2	28.31	28.34	28.2	4.7	0.9990
Tooth packing	30.64	28.08	29.24	31	6.0	0.7229
Tonge tingling	5.75	2.57	2.68	6.52	4.6	0.2493
Burnt	0.66[c]	25.69[a]	24.69[a]	14.12[b]	7.5	**< 0.0001**

[a] Means in the same row not labelled with the same letters are significantly different ($p < 0.05$); means are from two replicate samples.
[b] Least significance difference at $p = 0.05$.
[c] Probability, obtained from ANOVA, that there is a difference between the means.

sugars. In line with this, an interesting finding is that the assessors perceived that the samples with rare sugars and fructose had brown spices aroma, even though no spices or flavourings were added to the formulations. Potentially, compounds such as sotolone or furfural could be contributing to the perception of this scent (Erdem et al., 2023). Future work in the characterisation of volatile compounds in biscuits could also provide fundamental information when sucrose is replaced by other ingredients. It is worth noting that even though the browning reactions favour the formation of key aroma compounds, they also facilitate the generation of process contaminants (e.g., acrylamide and 5-hydroxymethylfurfural) (Nguyen et al., 2016); therefore, the assessment of these chemicals is relevant.

Regarding the mouthfeel or texture attributes, there were also significant differences in most of the attributes ($p > 0.05$), besides drying and tooth-packing. Contrary to the after-effect attributes of odour/taste, the mouthfeel after-effects did not present significant differences among samples. The main texture attribute of the biscuit, hardness of first bite, showed that the biscuits made with sucrose were perceived as harder, followed by fructose, allulose, and tagatose samples, which is different from the order of results observed in the instrumental analysis for sucrose, allulose, and fructose (see Table 1). The crunchy texture scored higher for the sucrose samples, which could be linked to the recrystallisation of the sucrose during the cooling process crispness (Pareyt et al., 2009). In terms of crunchiness, sucrose samples were followed by fructose, allulose, and to conclude tagatose, which could not be considered crunchy; this agrees with the increased fracturability linked to increased elasticity of the sample (see Table 1). A similar order in the perception of firmness and brittleness were obtained by Jeong et al. (2024). Perhaps, the biscuits elaborated with sucrose were perceived as harder because of the increased perception of crunchiness, demonstrating that cross-modal interactions that cannot be assessed with the instrumental analysis are happening.

Attributes appearing after masticating such as mouth coating, pasty, and body were significantly higher in the biscuits elaborated with fructose and rare sugars. To highlight, the samples with tagatose and allulose showed the highest mouth coating and body. Although the samples elaborated with rare sugars differed from the texture of the sucrose ones, it would be worth assessing the behaviour of these samples during oral processing, which could provide insights as to whether biscuits made with tagatose could be suitable for focus groups of consumers, for instance, with more preference for softer products.

4. Conclusion

This study demonstrated that rare sugars generated significant changes in almost all the properties of dough and biscuits compared with the control made with sucrose. The results also confirmed that the three epimers (fructose, allulose, tagatose) have different behaviours on the various physical and sensory properties of this food matrix, and for the first time, a comprehensive evaluation of tagatose on the thermal behaviour in wheat flour-sugar solution mixtures and rheological properties of dough was performed. In this sense, it was unveiled that the mutarotation of the epimers had a more prevalent impact, besides the MW and $N_{OH,s}$ on explaining the changes in starch gelatinisation temperature, dough consistency, and dough viscoelasticity, which subsequently reflected in biscuits moisture, dimensions and texture.

Overall, fructose and allulose had similar behaviour between each other, while tagatose produced the most distinct doughs and biscuits from the three epimers, and the most different doughs and biscuits from sucrose. For instance, among all sugars, tagatose doughs were the hardest and exhibited higher viscoelasticity (during all stages of heating). Besides, biscuits prepared with this sugar were the smallest and softest, with the highest moisture and a_w, with sensory results validating these findings. In this sense, doughs and biscuits prepared with allulose showed more similarities to the sucrose dough in terms of rheology (similar consistency and viscoelastic behaviour) and biscuit texture (similar hardness and fracturability) making it a more potential substitute over tagatose. Yet, it is suggested that partial substitution of sucrose with allulose could deliver biscuits with improved appearance, flavour, and texture (crunchiness), as some drawbacks of total replacement with all the epimers were the intense browning, low sweetness and reduced crunchiness. The present findings can be used to understand the implications of using allulose and tagatose in the reformulation of baked products like biscuits.

In this sense, although the thermal and rheological analyses helped to explain the differences in texture, moisture, and spreading, other analysis are necessary in order to understand the variations in colour and flavour/aroma. Since it is evident that browning reactions are augmented in biscuits with fructose and rare sugars, prospective studies in understanding the effect of rare sugars on flavour forming reactions and acrylamide formation in a matrix like biscuits is advised.

Financial declaration

This work was funded by the Biotechnology and Biological Sciences Research Council through a UKRI BBSRC Food Biosystems Doctoral Training Parthnership (DTP), grant number BB/T008776/1.

Ethical statement for sensory panel

Ethical review and approval were not necessary for this study as the study involved tasting standard commercial practices by a trained sensory panel that are employees and have consented to taste and rate food as part of their job. The trained panel work within the ethical and professional practices set out by the Institute of Food Science and Technology (IFST), 2020.

CRediT authorship contribution statement

Ana Maria Gomez-Betancur: Writing – original draft, Visualization, Validation, Resources. **Stella Lignou:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Beril Pinarli:** Visualization, Investigation, Formal analysis. **Victoria Norton:** Writing – review & editing, Formal analysis. **Julia Rodriguez-Garcia:** Conceptualization, Writing – review & editing, Supervision, Resources, Methodology.

Declaration of competing interest

Authors declare no competing interest and no non-financial competing interests, or other interests that might be perceived to influence the interpretation of the article.

Acknowledgements

The authors gratefully acknowledge Pedro Rivas-Ruiz from the Chemical Analysis Facility (CAF) at the University of Reading for their expert technical support and assistance in this work, and to Anderson Advanced Ingredients B.V. (Raalte, The Netherlands) for the suppling of the allSWEET® allulose and tagSWEET® tagatose used in this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fhfh.2025.100230.

Data availability

The data presented in this study are openly available in the University of Reading Research Data Archive at https://doi.org/10.17864/1947.001386. Supplementary material is available in the **Appendix A**.

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