ELSEVIER

Contents lists available at ScienceDirect

LWT



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

Roles of protein, starch and sugar in the texture of sorghum biscuits

Olumide A. Adedara¹, John R.N. Taylor

Department of Consumer and Food Sciences and Institute for Food, Nutrition and Well-being, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

ARTICLE INFO

Keywords: Breaking strength Gluten-free Kafirin Starch Sugar glass

ABSTRACT

This study investigated why biscuits made from sorghum flour have a similar texture to wheat biscuits despite the absence of gluten in sorghum dough. Electron microscopy revealed that the sorghum prolamin protein bodies remained intact in the sorghum biscuits and hence were unlikely to contribute to biscuit structure and texture. Polarized light microscopy showed that the starch granules in the sorghum biscuits were not gelatinized. Increasing dough water content increased the breaking strength and brittleness of sorghum biscuits. However, increasing the proportion of pre-gelatinized sorghum flour in the dough reduced the breaking strength of the sorghum biscuits, indicating that starch gelatinization weakened the biscuit structure. In contrast, increasing the sucrose content of the dough increased sorghum biscuit breaking strength and brittleness. At 20% sucrose (flour basis), the sorghum biscuits had similar breaking strength and brittleness to both Marie and sugar-snap wheat biscuits. DSC and X-ray diffractometry showed that the sugar glass embedded or enveloped the sorghum biscuit flour particles. It is concluded that this sugar glass matrix is responsible for the strength and cohesiveness of the sorghum biscuits.

1. Introduction

Sorghum is an alternative to wheat for the production of bakery products in sub-Saharan Africa as it is a widely cultivated cereal in the region, whereas wheat has to be mostly imported (FAOSTAT, 2017). Biscuits (also known as cookies) are a commonly consumed snack in Africa and worldwide (Ronquest-Ross et al., 2015). This is primarily because they are convenient, being ready-to-eat, and have high energy density and a long shelf life due to their low moisture content (only 1–5%).

The crucial importance of the viscoelastic and gas-holding properties of gluten, the protein complex formed during wheat flour dough mixing in leavened bread making is well known (Gallagher et al., 2004). However, in the case of biscuits it has been found that biscuits of consumer-acceptable sensory quality can made from sorghum flour, plus the normal of ingredients of sucrose and shortening only, despite its absence of gluten-forming proteins (Rao et al., 2016; Serrem et al., 2011). Descriptive sensory profiling of sorghum biscuits and wheat biscuits, which were made using the same recipe, revealed that the sorghum biscuits had a dry and crisp texture but were rather gritty, coarse and rough (Serrem et al., 2011). This was attributed to the fact that the sorghum flour used was coarser than the wheat flour.

Although biscuits of reasonable textural quality can be made from flours of many different types of gluten-free grains, including cereals, pseudocereals and legumes (Di Cairano et al., 2018) the science is not understood. This issue is not only important with regard to producing bakery products from gluten-free grains, it is also of relevance to the science and technology of wheat biscuit making. This is because despite considerable research, there is also not a consensus as to what is responsible for wheat biscuit texture. Gaines (1990), in a study of 64 wheat flours concluded that a gluten network was not (or was to a very limited extent) produced during mixing of sugar-snap biscuit doughs. However, the addition of the disulphide bond reducing agent dithioerythritol greatly affected dough consistency and spread. On the basis of this, the author suggested that the gliadin and glutenin proteins (the gluten precursor proteins) are functional during biscuit baking. In a review concerning sugar-snap biscuits, Pareyt and Delcour (2008) suggested that these proteins associate, even if few intra- and

Received 20 May 2020; Received in revised form 28 September 2020; Accepted 30 September 2020 Available online 3 October 2020 0023-6438/@ 2020 Elsevier Ltd. This is an open access article under the CC BV-NC-ND license (http:///

0023-6438/© 2020 Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Abbreviations: PLM, Polarized light microscopy; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; XRD, X-ray diffractometry. * Corresponding author.

E-mail address: john.taylor@up.ac.za (J.R.N. Taylor).

¹ Deceased.

https://doi.org/10.1016/j.lwt.2020.110323

Table 1

Effects of dough water content, raw flour:pregelatinized flour ratio and sucrose content and its state on sorghum biscuit stress, strain and brittleness. A. Dough water content,

B. Raw flour: Pregelatinized flour ratio (40% water content formulation, flour basis), C. Sucrose content and state added.

A. Water content of sorghum	Relative percentages of		Relative percentages of		For	ce (N)	Breaking strength		Strain	Brittleness
biscuit doughs (% flour basis	components in sorghum biscuit		components in sorghum				(Maximum stress) (kPa)		(%)	(Stress/Strain)
	doughs		biscuits ^c							
30	Flour: 56.3		Flour: 67.9		$11.19^{a} \pm$		$1.16^a\pm0.28$		$\textbf{2.83}^{b} \pm$	$0.42^a\pm0.13$
	Sucrose: 14.0		Sucrose: 17.0		1.58	8 ^{1,2}			0.30	
	Oil: 12.5		Oil: 15.1							
	Water: 16.9					,	,			,
40	Flour: 53.2		Flour: 67.9		15.7	74 ^b ±	$1.77^{b} \pm 0.13$	5	$2.78^{\text{b}} \pm$	$0.62^{\text{b}}\pm0.14$
	Sucrose: 13.3		Sucrose: 17.0		1.29	9			0.26	
	Oil: 11.9		Oil: 15.1							
	Water: 21.3					_			L	
50	Flour: 50.5		Flour: 67.9		26.4	45 ^c ±	$2.75^{\circ} \pm 0.59$)	$2.83^{\text{D}} \pm$	$1.00^{\circ} \pm 0.25$
	Sucrose: 12.6		Sucrose: 17.0		2.11	1			0.30	
	011: 11.3		011: 15.1							
a.e. 1 1	Water: 25.3				1.0	- ob	1 ooah o 1	0	0.003	o cch i o oo
Marie wheat biscuits					16.5	$50^{0} \pm$	$1.38^{ab} \pm 0.1$	9	2.00 ^a ±	$0.66^{9} \pm 0.09$
0 1 . 1					2.25		1 0 03 + 0 14		0.00	0.003 + 0.04
Sugar-snap wheat discuits					24.5	57°±	$1.28^{-} \pm 0.12$	2	3.33° ±	$0.38^{\circ} \pm 0.04$
					2.30)			0.00	
B. Raw flour: Pre-gelatinized flour ratio of sorghum biscuit doughs Force (N) Breaking strength (Maximum stress) (kPa) Strain (%) Brittleness (Stress/St									ess (Stress/Strain)	
100:0			$15.74^{d} \pm 1.29^{1} \\$	$1.77^{\rm d}\pm0.15$				$2.46^{\mathrm{b}}\pm0.3$	3 0.73 ^d ±	0.13
80:20			$12.00^{\text{c}}\pm1.73$	$1.41^{c}\pm0.21$				$2.78^{\rm c}\pm0.2$	6 0.52 ^c ±	0.10
60:40			$8.54^{b}\pm1.18$	$0.99^b\pm0.24$				$2.83^{c}\pm0.3$	0 0.36 ^b ±	0.11
0:100			$3.09^{a}\pm0.92$	$0.38^{a}\pm0.14$				$2.78^{ m c}\pm0.2$	6 0.14 ^a ±	0.06
Marie wheat biscuits			$16.50^{\rm d}\pm2.25$	$1.38^{c}\pm0.19$				$2.00^{\mathrm{a}}\pm0.0$	0 0.66 ^d ±	0.09
Sugar-snap wheat biscuits			$\textbf{24.57}^{\text{e}} \pm \textbf{2.30}$	$1.28^{c}\pm0.12$				$3.33^{d} \pm 0.0$	0 0.38 ^b ±	0.04
C. Sucrose content of	Relative percentages of	Relativ	ve percentages of	State sucr	ose	Force (N)	Breakin	g strength	Strain	Brittleness
sorghum biscuit doughs	components in sorghum	components in sorphur		added	000	10100 (11)	(Maxim	(Maximum stress) (kPa)		(Stress/Strain)
(% flour basis)	biscuit doughs biscuite		s ³	uuucu			(kPa)			(ou cos, ou uni)
0	Elour 62.2	Tlaum	01.7	Not		0.748	0.408	0.07	2 opb	0.1.43 + 0.04
0	FIGUE: 03.3	FIGUE: 81.7		not		$3.74^{\circ} \pm$	$0.40^{\circ} \pm 0.07$		2.83 ±	$0.14^{\circ} \pm 0.04^{\circ}$
	Sucrose: 0	Sucros	22	applicable	e	0.37			0.30	
	011. 14.1 Water: 22.1	011. 16	5.5							
10	Flour: 50 5	Flour	75.6	Dra		7 17 ^b ⊥	0 70 ^b +	0.17	2 83 ^b ⊥	$0.20^{abc} \pm 0.08$
10	riour. 59.5		73.0	DIy		0.70	0.79 ±	0.79 ± 0.17		0.29 ± 0.08
	Sucrose: 6.0	Sucros	e 76	Pre-dissol	Pre-dissolved		0 75 ^{ab} -	$0.75^{ab} + 0.12$		$0.27^{ab} + 0.06$
	Sucrose. 0.0	Jucióa		110-013301	vcu	0.44	0.75	0.12	2.70 ±	0.27 ± 0.00
	Oil: 13.3	Oil: 16	5.8							
	Water: 20.8									
20	Flour: 56.2 F		70.3	Drv		$11.96^{\circ} \pm$	$1.26^{\rm c} \pm 0.20$		$2.83^{ m b} \pm$	$0.46^{ m cd}\pm0.10$
				,		1.73			0.30	
	Sucrose: 11.2	Sucros	e: 14.1	Pre-dissol	ved	$12.70^{c} \pm$	$1.38^{ m c}$ \pm	0.38	$2.83^{b} \pm$	$0.50^{ m de}\pm0.16$
						1.93			0.30	
	Oil: 12.5	Oil: 15	5.7							
	Water: 19.7									
30	Flour: 53.2	Flour:	65.5	Dry		$24.24^{e} \pm$	2.61^{d} \pm	0.54	$2.83^{ m b}$ \pm	$0.95^{\rm f}\pm0.26$
				-		2.16			0.30	
	Sucrose: 16.0	Sucros	e: 19.7	Pre-dissol	ved	$22.39^{e} \pm$	2.41^{d} \pm	0.52	$2.83^{ m b}$ \pm	$0.87^{\rm f}\pm0.24$
						1.80			0.30	
	Oil: 11.9	Oil: 14	1.7							
	Water: 18.6									
Marie wheat biscuits				Not		$16.50^{d} \pm$	$1.38^{c} \pm$	0.19	$2.00^{a} ~\pm$	$\textbf{0.66}^{e} \pm \textbf{0.09}$
				applicable	е	2.25			0.00	
Sugar-snap wheat biscuits				Not		$\textbf{24.57}^{\text{e}} \pm$	$1.28^{ m c}$ \pm	0.12	$3.33^{c} \ \pm$	$0.38^{bcd}\pm0.04$
				applicable	e	2.30			0.00	

¹Mean \pm Standard deviation of twelve biscuits (n = 12).

²Values in a column with different superscript differ significantly (p < 0.05).

³Calculated on the basis of zero percent moisture in the biscuits.

intermolecular bonds are formed, and that sugar-snap biscuit hardness is "sensitive" to increased association of the proteins. That wheat proteins are important in biscuit quality seemed to be confirmed by Maache-R-ezzoug et al. (1998) who found that varying the flour protein content between 14 and 20% resulted in major changes in dough rheological properties and biscuit dimensions and texture. However, Chevallier et al. (2000) proposed, on the basis of dough and biscuit macro- and microscopic data, that wheat biscuit structure results from sugars melting during baking and forming bridges between protein aggregates

and lipids. On cooling, a continuous glassy phase of sugars embeds these components. This in turn embeds intact or little damaged starch granules. This is slight contrast to the conclusion of Baltsavias et al. (1999) that irrespective of wheat biscuit composition, the matrix whether it be gluten, sucrose or both is in a glassy state. In further contrast, Gallagher et al. (2004) stated that due to the minimal formation of a gluten network in wheat biscuits, their texture is primarily due to starch gelatinization and super-cooled sugar. These differing views on the roles of gluten and other components in wheat biscuit texture may in part be a

reflection of the type of wheat biscuit being investigated as biscuits differ in their formulations. Concerning sugar content alone, two very different types can be distinguished: semi-sweet biscuits containing 17–25% sugar (flour basis), for example Marie-type biscuits, which exhibit minimal dough spreading during baking, and sugar-snap cookies containing approx. 60% sugar (flour basis) where the dough expands considerably during baking (Pareyt & Delcour, 2008).

With the aim of establishing why sorghum biscuits have similar texture to wheat biscuits, the roles of the kafirin prolamin protein, gelatinized starch and sugar were investigated in semi-sweet type sorghum biscuits. Their functionality was compared to commercial semi-sweet wheat biscuits and laboratory-prepared standard recipe sugar-snap wheat biscuits, representing extremes in wheat biscuit type with respect to sugar content and dough behaviour.

2. Materials and methods

2.1. Materials

These were all purchased from a local supermarket in South Africa: Sorghum meal, fine grade (approx. 90% extraction rate) produced from red, non-tannin cultivars, total phenolic content 90 mg catechin equiv./ 100 g (as is basis) (Monati Super Mabela brand, RCL Foods, Westville, South Africa); Wheat flour, cake flour grade (10.4% protein, as is) (Snowflake brand, Premier Foods, Waterfall City, South Africa); Other biscuit ingredients, namely: cane sugar (sucrose), sunflower oil (sorghum biscuits), vegetable oil baking fat (sugar-snap wheat biscuits), baking powder; Commercial semi-sweet, Marie-type wheat biscuits (Bakers Blue Label brand, National Brands, Bryanston, South Africa), made with unhydrogenated vegetable oil.

2.2. Methods

2.2.1. Biscuit making

2.2.1.1. Sorghum biscuits. The sorghum meal was milled into a flour using a hammer mill (Drotsky, Alberton, South Africa) fitted with a 500 μ m opening screen. Semi-sweet sorghum biscuits were prepared according to Serrem et al. (2000). In brief, the standard formulation was 150 g flour, 37.5 g sucrose (i.e. 25% on a flour basis), 33.5 g sunflower oil (22% flour basis), 52.5 g water (35% flour basis) and 1 g baking powder. Aside from the fact we have found sunflower oil to be an effective shortening in the production of sorghum biscuits, it was used in this present work so as to avoid the complication of the role of a solid shortening in biscuit gross texture.

All ingredients were manually mixed into a dough, which was rolled out on a baking tray to a uniform thickness of 4 mm. Manual mixing was used as it was more effective than mechanical dough mixing at the experimental scale used. Dough pieces of dimensions 50×25 mm were cut out manually using a rectangular galvanized steel dough cutter. The dough pieces were baked in a convection oven at 463 K for 18 ± 2 min. After baking, the biscuits were cooled to ambient temperature (298 K) before packing into polyethylene zip-lock bags and stored at 253 K.

Sorghum biscuits were also prepared with different dough compositions: Different water levels (30%, 40% and 50% flour basis); Replacement of raw flour with different proportions of pregelatinized flour (20%, 40% and 100% flour basis) produced by wet cooking the flour for 3 min with 60 g water (40% total flour basis) and replacing the weight of water lost by evaporation; Different sucrose levels (0%, 10%, 20%, 30% flour basis) added dry (undissolved, as in the standard formulation) or pre-dissolved in water (60 g, 40% flour basis). When the water and sucrose levels were altered the amounts of the other ingredients were not adjusted. Hence, with increasing the dough water level there were small decreases in the proportions of the other components of the doughs but not in the biscuits (Table 1A), and with increasing the sucrose content of the doughs, there were decreases in the proportions of the other components in the doughs and in the biscuits (Table 1C). All biscuits, irrespective of their formulae were fully baked so as to eliminate the effect of moisture content on the texture of the biscuits. The typical aroma of baked products was used as indication that the biscuits were fully baked.

2.2.1.2. Wheat biscuits. Sugar-snap wheat biscuits, 58% sugar and 28% fat (flour basis), were made according to AACC approved method 10-50D (AACC, 2000).

2.3. Analyses

2.3.1. Stress and strain

Biscuit maximum stress and strain were determined using a TA.XT2 series texture analyser (Stable Microsystems, Godalming, UK) fitted with a 3-point bend rig attachment (HDP/3 PB), with a 30 mm distance between the supports. The maximum peak force obtained with a single compression was measured. The cross-head test speed was 3.0 mm/s with compression to a distance of 10 mm. The threshold force for the test was 0.49 N. Twelve individual biscuits per treatment were measured. Biscuit maximum stress (breaking strength) and strain (% extensibility) were calculated as follows:

 $\sigma = 3FL/2bh^2$ $\varepsilon = 6bh/L^2$ (Baltsavias et al., 1997)

 σ = Stress at midpoint (MPa), ϵ = Strain, F = force at the beam centre in Newtons, L = distance between the supports (mm), b = biscuit width (mm), h = biscuit thickness (mm).

2.3.2. Electron microscopy

The biscuits were crushed into crumbs using a pestle and mortar and then defatted with hexane. For scanning electron microscopy (SEM), the defatted biscuit crumbs were mounted on aluminium stubs using carbon tape and the crumbs coated with carbon. Samples were analysed using a Zeiss 540 Crossbeam SEM (Oberkochen, Germany) at 1 kV. For transmission electron microscopy (TEM), the defatted biscuit crumbs were fixed in glutaraldehyde, rinsed then dehydrated in an ethanol series. They were further post-fixed with aqueous osmium tetroxide and the infiltrated in an epoxy resin series. Slices (100 nm) were stained in uranyl acetate. Samples were viewed using a Philips CM10 TEM (Eindhoven, The Netherlands) at 80 kV.

2.3.3. Polarized light microscopy

Crushed undefatted biscuits, crystalline sucrose and sucrose glass were milled into a flour using an air-cooled, knife-type laboratory mill. A sugar glass was prepared by making a saturated solution of the finely milled sucrose plus corn syrup at 423 K (Levenson & Hartel, 2005). A polarized light microscope (Zeiss Axio Imager.Alm, Jena, Germany) was used to observe the interactions between in the sucrose and the other components of the biscuits. Biscuit flour (50 mg) was weighed into Eppendorf tubes and dispersed in 0.5 ml ethanol by vortexing. The samples were viewed at 200x magnification.

2.3.4. Stereomicroscopy

The sorghum and wheat biscuits were broken by hand and the facture surface viewed using a stereomicroscope (Zeiss Discovery V20, Jena, Germany) with a field of view 3.5 mm, 1.8 μ m resolution and 64 μ m depth of field at a magnification of 65x.

2.3.5. Differential scanning calorimetry (DSC)

Thermal analysis was conducted using a Mettler Toledo HP DSC 827e DSC (Greifensee, Switzerland). Biscuit flours (10–15 mg) were accurately weighed into DSC pans, hermetically sealed and scanned from 298 to 493 K at 10 K/min. The procedure was repeated with thoroughly mixed 30% (biscuit basis) finely milled crystalline sucrose and biscuit flour.



Bar = $10 \mu m$.



Bar = 1 μ m

В

Fig. 1. Electron microscopy of the particle structure of wheat and sorghum biscuits. a) Marie wheat biscuit, b) Sorghum biscuit, c) Sugar-snap wheat biscuit. A. Scanning electron microscopy - SG = Starch granules, Solid arrows = Sheet-like matrix, Dotted arrows = Probable protein bodies B. Transmission electron microscopy - PM = Protein matrix, SG = Starch granules, SLM = Sheet-like matrix.

2.3.6. X-ray diffractometry (XRD)

Milled sucrose and sugar glass, sorghum and wheat flours and the biscuit flours were dried over silica gel in a desiccator for 120 h. Samples were analysed using a PANalytical X'Pert Pro powder diffractometer (Almelo, The Netherlands) in θ - θ configuration with a X'Celerator detector and variable divergence- and fixed receiving slits with Fe filtered Co-K α radiation ($\lambda = 1.789$ Å). The diffraction phases were identified using X'Pert Highscore plus software.

2.3.7. Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA). All experiments were repeated. A minimum of 12 biscuits per treatment were evaluated for texture and means were compared at p = 0.05 using the Tukey Honest Significance Difference test (HSD).

3. Results and discussion

3.1. State and role of sorghum kafirin protein in sorghum biscuits

SEM showed that the starch granules were intact in both the defatted sorghum and wheat biscuits. (Fig. 1A). A continuous sheet-like matrix (solid arrows) largely enveloped the starch granules in the sugar-snap and semi-sweet Marie-type wheat biscuits. In contrast, in the sorghum biscuits there was mainly aggregations of starch granules with a few much smaller spherical objects (dotted arrows), which were probably protein bodies. TEM revealed that in the sorghum biscuits where a

protein matrix was observed around the starch granules, presumably flour particles from the protein-rich corneous endosperm, the kafirin protein bodies appeared to be intact (Fig. 1Bb). This indicates that the kafirin did not participate in dough formation and therefore could not have contributed to the texture of sorghum biscuits. Kafirins are noted for being considerably more hydrophobic and inert than the gluten proteins and in fact the kafirin protein bodies remain intact, even when subjected to hydrothermal treatment (Duodu et al., 2002) or break apart during dough mixing (Goodall et al., 2012). In the case of the wheat biscuits, the sheet-like matrix had voids in it (dark areas) (Fig. 1Ba,c). Since the biscuits had been extracted with hexane and treated using an aqueous ethanol series during fixing for TEM, it is likely that the voids represent the location of the lipids and sucrose removed during sample preparation. The remaining matrix would thus comprise predominantly gluten. This is in line with the theories of Doescher et al. (1987) and Chevallier et al. (2000). The former authors proposed that during baking the gluten undergoes a glass transition to form a continuous matrix. The latter authors theorised that protein and lipid aggregates would be embedded in the sugar phase. The matrix in the sugar-snap biscuits was less continuous than that in the Marie biscuits, which was probably due to its much higher sugar content than Marie biscuits.

3.2. State of starch and effect of starch gelatinization in sorghum biscuits

Polarized light microscopy (PLM) showed that the starch in the sorghum biscuits was in the ungelatinized, semi-crystalline form as the



Fig. 2. Polarized light microscopy of the crumb structure of sorghum biscuits. a) Crystalline sucrose, b) Sugar glass, c) Sorghum biscuit made without added sucrose, d) Sorghum biscuit made with 10% added sucrose, e) Sorghum biscuit made with 20% sucrose. SG = Starch granules, Bar = $20 \mu m$, Arrows indicate sugar glass.



Fig. 3. Stereomicroscopy showing the effects of different dough water contents on the crumb structure of sorghum biscuits when compared to wheat biscuits. Sorghum biscuits - dough water content: a) 30%, b) 40%, c) 50%, d) Marie wheat biscuit, e) Sugar-snap wheat biscuit. Bar = 0.5 mm, Arrows indicate strands.

starch granules exhibited birefringence crosses, irrespective of the proportion of sucrose in the biscuit formulation, from 0% (flour basis) to 30% (Fig. 2).

With the aim of increasing water availability to facilitate starch gelatinization, the effect of dough water content on sorghum biscuit texture was studied over the range 30% water (the minimum that produced a cohesive dough) to 50% (which produced a sloppy dough). Biscuits made with 30% water were similar ($p \ge 0.05$) in stress (breaking strength) to the wheat Marie and sugar-snap biscuits and similar in stress/strain (brittleness) to the sugar-snap biscuits (Table 1A). Both the 30% water sorghum and the sugar-snap wheat biscuits were less brittle than the Marie biscuit (p < 0.05). An increase in dough water content increased sorghum biscuit breaking strength and brittleness from 1.16 kPa to 2.75 kPa and from 0.42 to 1.00 at 30% and 50% water,

respectively. The increases in biscuit breaking strength and brittleness with increasing dough water content were probably due to increased hydration of the flour particles enabling better infiltration of the dissolved sucrose. This would result in a more uniform and hence stronger sugar glass matrix (see Section 3.3). Manohar and Rao (1999) also observed an increase in the breaking strength of semi-sweet wheat biscuits with increased dough water content, but did not offer and explanation as to the cause. With 50% dough water, the sorghum biscuits were both stronger and more brittle (p < 0.05) than either of the wheat biscuit types.

Stereomicroscopy showed that the crumb structures of three sorghum biscuit formulations prepared with three different water levels and the sugar-snap and semi-sweet biscuits were all essentially the same. They comprised flour particles adhered to each other to form a



Fig. 4. DSC thermogramsof sorghum and wheat biscuits. (a) Sorghum biscuit made without sugar, (b) Sorghum biscuit*, (c) Sorghum biscuit* plus 25% crystalline sucrose, (d) Marie wheat biscuit, (e) Marie wheat biscuit plus 25% crystalline sucrose, (f) Sugar-snap wheat biscuit, (g) Sugar-snap wheat biscuit plus 25% crystalline sucrose, (h) Sugar glass, (i) Crystalline sucrose. *Sorghum biscuit prepared with 30% sugar added during dough formulation. Arrows indicate melting endotherm of crystalline sucrose.

continuous structure (Fig. 3). The flour particles in the sorghum biscuits were larger (Fig. 3a-c) than in the Marie and sugar-snap wheat biscuits (Fig. 3d and e), as would be expected since the sorghum flour was coarser than wheat flour. Also, the surface of the sorghum biscuit particles was smoother and with few long very thin strands on them (indicated by arrows). These strands may have comprised starch granules entrapped in sugar glass or gelatinized and partially solubilized starch. However, there was no evident trend in the number of starch granule strands in the sorghum biscuits with increasing level of dough water, which was expected with starch gelatinization. In wheat biscuits, starch gelatinization is generally limited (Abboud & Hoseney, 1984) and this been proposed to be due to the excess of sugar competing with the starch for the limited amount of water (Mammat Abu Hardan & Hill, 2010). However, with sorghum biscuits this does not seem to be necessarily the case as PLM revealed that the starch granules were not gelatinized even when biscuits were made without sugar (Fig. 2c). The absence of significant gelatinization in the standard recipe sorghum biscuits may have been due to the relatively low proportion of water in the dough (35% flour basis) compared to bread doughs. This is in general agreement with the conclusion of Baltsavias et al. (1999) who studied the fracture properties of wheat short-dough biscuits.

Substituting raw sorghum flour with pre-gelatinized flour resulted in a severe reduction in sorghum biscuit breaking strength, from 1.77 kPa with 100% raw flour biscuits to 0.37 kPa in 100% gelatinized flour biscuits (Table 1B). Taken together, these findings clearly show that starch gelatinization was not responsible for the similar texture of sorghum biscuits to wheat biscuits.

3.3. State and role of sugar in sorghum biscuits

Increasing the sucrose content of the sorghum doughs resulted in a dramatic increase in biscuit breaking strength (Table 1C). With inclusion of dry sucrose in the dough, biscuit strength increased from 0.40 kPa with none added, to 0.79 kPa with 10% sucrose inclusion (flour basis), to 2.61 kPa with 30% inclusion. There was a concomitant increase in biscuit brittleness, from 0.29 (10% sucrose) to 0.95 (30% sucrose). It is unlikely that the reduction in the proportions of the sorghum flour and vegetable oil by the higher content of sucrose had a significant influence on the changes in biscuit strength as a 30% (flour basis) dough sugar content is not substantially higher than the normal range for wheat semi-sweet biscuits of 18–25% (Pareyt & Delcour, 2008).

With 20% sucrose inclusion the sorghum biscuits had similar



Fig. 5. X-ray diffractometry diagrams of crystalline sucrose, sugar glass and the centre and top surfaces of sorghum biscuits, wheat Marie biscuits and wheat sugarsnap biscuits.

breaking strength and brittleness (p \geq 0.05) to the wheat Marie and sugar-snap biscuits.

Notably, the effects of sucrose inclusion were the same ($p \ge 0.05$) irrespective of whether the sucrose was added dry or pre-dissolved. This indicates that the added dry sucrose completely dissolved in the dough aqueous phase during dough making and biscuit baking. With semi-sweet wheat biscuits, Manohar and Rao (1997) observed different effects on semi-sweet wheat biscuit breaking strength; an increase with a low gluten flour and decrease with higher gluten flour. With wheat sugar-snap biscuits, a small increase in breaking strength was observed as the sugar content was increased from 17.6% to 31.2% (dough basis) (Paryet et al., 2009).

With DSC thermal analysis, crystalline sucrose gave an endotherm with a peak temperature of 193.2 °C (Fig. 4i). This endotherm temperature range is consistent with the range of literature values for the melting temperature of sucrose of 180–192 °C reported by Hurtta et al. (2004). In contrast, there was not an endotherm with the sugar glass (Fig. 4h), nor with the sorghum biscuit formulations (no added sucrose or 30% sucrose added during dough formulation) (Fig. 4a and b), nor with the wheat Marie and sugar-snap biscuits (Fig. 4d,f). However, when crystalline sucrose was mixed with the 30% sucrose sorghum biscuit (Fig. 4c) and the two wheat biscuit types (Fig. 4e,g), all three exhibited an endotherm in the peak temperature range of 183.8–186.5 °C. The lower endotherm temperature for the biscuits with added crystalline sucrose compared to sucrose alone is probably due to the interfering effect of the other carbohydrates present in the biscuits. The absence of an endotherm in the sorghum and wheat biscuits and its presence when

crystalline sucrose was added shows that the sugar in the sorghum and wheat biscuits was predominantly the glassy state. This would explain why crystalline sucrose or dissolved sucrose addition both had identical effects on sorghum biscuit texture (Table 1C).

XRD was applied to samples from the centre and top surface of the sorghum and wheat biscuits with the aim of establishing whether there were subtle differences in sucrose state between the biscuit types or within the biscuits themselves (Fig. 5). The graphs of the sorghum, semisweet and sugar-snap wheat biscuits were all similar to that of the sugar glass, confirming that the sucrose in the biscuits was predominantly in a glassy state. However, the centre of the sugar-snap wheat biscuit gave one clear diffraction peak and several peaks with the top surface. There were also two peaks with the top surface of the sorghum biscuits. These peaks corresponded in Bragg angle with certain of the crystalline sucrose diffraction peaks, showing that these biscuits contained a small proportion of crystalline sucrose. There were no clear peaks from the sorghum biscuit centre or from the centre or surface of the wheat semisweet biscuits. The observed sugar crystallinity of the wheat biscuits is consistent with previous work. Sugar crystals have been observed in the centre of sugar-snap wheat biscuits (Hoseney & Rogers, 1994) and sucrose X-ray diffraction peaks have been observed from the top surface of wheat shortbread biscuits but not from the centre (Chevallier et al. (2000). Thus, it appears that in terms of sucrose crystallinity, the semi-sweet sorghum biscuits were intermediate between the semi-sweet and sugar-snap wheat biscuits.

PLM provided information about the distribution of the sugar glass in the sorghum biscuit crumb (Fig. 2). With biscuits made with 10% and

20% sucrose, many of the crumb particles were embedded in a matrix of sugar glass, which appeared to be infiltrated between the starch granules (Fig. 2de). With biscuits made with 30% sucrose, some of the crumb particles were completely enveloped in a sugar glass. Chevallier et al. (2000) proposed the sugar melt could form a bridge between starch-protein particles in wheat biscuits. This also seems to be the case in sorghum biscuits.

4. Conclusions

This study revealed that the sucrose in the biscuit formulation is primarily responsible for the similar texture of sorghum biscuits to that of wheat biscuits and that neither the kafirin prolamin proteins nor the starch play a significant role. Further, sorghum biscuits of similar texture to semi-sweet or sugar-snap wheat biscuits can be produced using solely sorghum flour plus normal biscuit ingredients by adjusting the level of sucrose and water used in the dough formulation. During baking, the sucrose melts and infiltrates into the dough. On cooling, it forms a glass that embeds and envelops the flour particles. This sugar glass matrix is responsible for the strength and cohesiveness of the sorghum biscuits. Furthermore, it is suggested that the greater breaking strength of sorghum biscuits with increasing dough water content is due to better infiltration of the sugar glass into the flour particles. By implication, the findings of this study support the contention that a sugar glass is primarily responsible for the gross crumb structure and physical texture of hard wheat biscuits.

CRediT authorship contribution statement

Olumide A. Adedara: Investigation, Data curation, Writing - original draft, manuscript. **John R.N. Taylor:** Supervision, Conceptualization, Methodology, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Alan Hall of the Laboratory for Microscopy and Microanalysis, and Antoinette Buys of the Electron Microscopy Unit, Department of Anatomy and Physiology, University of Pretoria. The South African National Research Foundation for funding (Grant No. 85762).

References

AACC. (2000). Approved methods of the AACC. In *Baking quality of cookie flour, Method* 10-50D (10th ed.). St. Paul, MN: American Association of Cereal Chemists.

- Abboud, A. M., & Hoseney, R. C. (1984). Differential scanning calorimetry of sugar cookies and cookie doughs. *Cereal Chemistry*, 61, 34–37.
- Baltsavias, A., Jurgens, A., & Van Vliet, T. (1997). Rheological properties of short doughs at small deformation. *Journal of Cereal Science*, 26, 289–300.
- Baltsavias, A., Jurgens, A., & Van Vliet, T. (1999). Fracture properties of short-dough biscuits: Effect of composition. *Journal of Cereal Science*, 29, 235–244.
- Chevallier, S., Colonna, P., Buleon, A., & Della Valle, G. (2000). Physicochemical behaviours of sugars, lipids, and gluten in short dough and biscuit. *Journal of Agricultural and Food Chemistry*, 48, 1322–1326.
- Di Cairano, M., Galgano, F., Tolve, R., Caruso, M. C., & Condelli, N. (2018). Focus on gluten free biscuits: Ingredients and issues. *Trends in Food Science & Technology*, 81, 203–212.
- Doescher, L. C., Hoseney, R. C., & Milliken, G. A. (1987). A mechanism for cookie dough setting. *Cereal Chemistry*, 64, 158–163.
- Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., & Taylor, J. R. N. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science*, 35, 161–174.
- FAOSTAT. (2017). New food balances. http://www.fao.org/faostat/en/#data/FBS/. (Accessed 14 May 2020).
- Gaines, C. S. (1990). Influence of chemical and physical modification of soft wheat protein on sugar-snap cookie dough consistency, cookie size, and hardness. *Cereal Chemistry*, 67, 73–77.
- Gallagher, E., Gormley, T. R., & Arendt, E. K. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science & Technology*, 15, 143–152.
- Goodall, M. A., Campanella, O. H., Ejeta, G., & Hamaker, B. R. (2012). Grain of high digestible, high lysine (HDHL) sorghum contains kafirins which enhance the protein network of composite dough and bread. *Journal of Cereal Science*, 56, 352–357.
- Hoseney, R. C., & Rogers, D. E. (1994). Mechanism of sugar functionality in cookies. In H. H. Faridi (Ed.), *The science of cookies and cracker production* (pp. 203–226). New York: Chapman and Hall.
- Hurtta, M., Pitkänen, I., & Knuutinen, J. (2004). Melting behaviour of D-sucrose, Dglucose and D-fructose. Carbohydrate Research, 33, 2267–2273.
- Levenson, D. A., & Hartel, R. W. (2005). Nucleation of amorphous sucrose-corn syrup mixtures. *Journal of Food Engineering*, 69, 9–15.
- Maache-Rezzoug, Z., Bouvier, J., Allaf, K., & Patras, C. (1998). Effect of principal ingredients on rheological behaviour of biscuit dough and on quality of biscuits. *Journal of Food Engineering*, 35, 23–42.
- Mamat, H., Abu Hardan, M. O., & Hill, S. E. (2010). Physicochemical properties of commercial semi-sweet biscuit. Food Chemistry, 121, 1029–1038.
- Manohar, R. S., & Rao, P. H. (1997). Effect of sugars on the rheological characteristics of biscuit dough and quality of biscuits. *Journal of the Science of Food and Agriculture*, 75, 383–390.
- Manohar, R. S., & Rao, P. H. (1999). Effect of water on the rheological characteristics of biscuit dough and the quality of biscuit. *European Food Research and Technology*, 209, 281–285.
- Pareyt, B., & Delcour, J. A. (2008). The role of wheat flour constituents, sugar and fat in low moisture cereal based products: A review on sugar-snap cookies. *Critical Reviews* in Food Science and Nutrition, 48, 824–839.
- Pareyt, B., Talhaoui, F., Kerckhofs, G., Brijs, K., Goesaert, H., Wevers, M., & Delcour, J. A. (2009). The role of sugar and fat in sugar-snap cookies: Structural and textural properties. *Journal of Food Engineering*, 90, 400–408.
- Rao, B. D., Anis, M., Kalpana, K., Sunooj, K. V., Patil, J. V., & Ganesh, T. (2016). Influence of milling methods and particle size on hydration properties of sorghum flour and quality of sorghum biscuits. *LWT-Food Science and Technology*, 67, 8–13.
- Ronquest-Ross, L. C., Vink, N., & Sigge, G. O. (2015). Food consumption changes in South Africa since 1994. South African Journal of Science, 111, 1–12.
- Serrem, C. A., de Kock, H. L., & Taylor, J. R. N. (2011). Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. *International Journal of Food Science and Technology*, 46, 74–83.