



Influence of short chain fructo-oligosaccharide (SC-FOS) on the dough rheological, microstructural properties and, bread quality during storage

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ABSTRACT

Objective of this study was to understand the impact of short chain fructo-oligosaccharides (SC-FOS) on the dough rheological characteristics and bread quality. SC-FOS was used in place of sucrose and studied for dough rheology, bread making and sensory characteristics. Farinograph, extensograph, amylograph and rheometer were used to evaluate the rheological characteristics. The interaction of SC-FOS with starch was explored from a structural perspective, as well as how SC-FOS influences to keep the bread softer over a period of time. For mechanistic insight, FT-IR (Fourier transform infrared spectroscopy), PXRD (Powder X-ray diffraction), and SEM (Scanning electron microscope) were used to investigate the structural changes. Starch crystallization through molecular and supramolecular reorganization primarily dictates the texture of bakery products, particularly the firmness of product like bread where moisture content is higher; SC-FOS can successfully slow down the crystallization of starch and yield softer product during a shelf life of 7 d. Bread with SC-FOS has shown better retention of water and modulating mobility of water. As a result, incorporating SC-FOS to bread has led to a positive outcome, with improved quality and an excellent sugar replacer.

1. Introduction

Bread is a staple and a prominent food prepared by baking the dough of wheat flour and water along with other ingredients. Sugar in the formulation facilitates absorption of water, keeps the product soft and tender, acts as a substrate for yeast to raise faster and function as a preservative by keeping it fresh for a longer period apart from the crust caramelization (Yadav, Patki, Sharma, & Bawa, 2009). Fructo-oligosaccharide, a sugar replacer and a functional ingredient would enhance the nutritional quality of the product. Short chain fructooligosaccharide (SC-FOS), a prebiotic fiber and a natural sweetener can replace the sugar levels up to 40 g/100g and can be claimed for digestive health and blood sugar management (Sabater-Molina, & M. Larqué, 2009). The moisture content and water activity in breads were higher during storage wherein the retardation of spoilage including the texture staling was slower and elicited a low glycemic response beneficial to all consumers (Storey, Lee, Bornet, & Brouns, 2007).

Fructo-oligosaccharides are chains of plant sugars taken from asparagus, Jerusalem artichokes and soybeans or can be produced using natural ingredients like sugar.

SC-FOS are a mixture of oligosaccharides produced by the enzymatic process, consists of glucose linked to fructose units through β -(1,2) (Hirayama, Sumi, & Hidaka, 1989). SC-FOS occur in a number of edible plants, such as chicory, onions, garlic, banana, asparagus, wheat having linear fructose oligomers with a degree of polymerization ranging from $n = 1$ to 5 (oligosaccharides) with a terminal glucose molecule. SC-FOS, to a large extent, escape digestion in the human upper intestine (Molis et al., 1996) and reach the colon and get fermented to lactate, short chain fatty acids (acetate, propionate and butyrate) and gas. Ingestion of SC-FOS for a period of 4 wk at 20g per day had no effect on glucose and lipid metabolism in type 2 diabetics. According to the presentation made by the scientists in the Institute of Food Technologists, Chicago in 2013, SC-FOS are low-calorie, non-digestible carbohydrates improve the food taste and texture while aiding immunity and bone health help in the

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growth and balance of important bacteria in the digestive track. Also, short-chain fructooligosaccharides (SC-FOS) are fermentable fibers that can be added to foods to increase satiety which is linked to colonic fermentation indicating that the SC-FOS on fermentation did not enhance the hunger (Hess, Birkett, Thomas, & Slavin, 2011). Priyali et al. (2021) reported that liquid or solid forms of SC-FOS did not increase the postprandial glucose and insulin levels when consumed alone or when added to available carbohydrates as it is not digested by the enzymes in the human small-intestine.

For several years, food scientists have worked to maintain a soft texture and shelf stable bread to the customer. Firming of baked goods is a process linked to retrogradation of starch along with other complex mechanism related to staling. This has drawn tremendous attention as it affects the quality of foods, primarily during storage. It is being widely believed that, bread staling is mainly due to retrogradation of starch (Vigneshwari & Manonmani, 2020). For the baked product, moisture and sugar amount may commonly influence the retrogradation phenomena. The effect of SC-FOS on the rheological characteristics of wheat flour dough, bread quality, and storage stability was the focus of this study. The research also used fundamental and empirical rheology to investigate the interactions of SC-FOS on wheat flour dough. Interaction of starch with SC-FOS studied from structural aspects and how SC-FOS influenced microstructure to keep bread product soft overtime is also looked into.

2. Materials and methods

2.1. Raw materials

Refined wheat flour procured from local market was characterized for physico-chemical parameters according to standard AACC, 2010 procedures and the flour had ash content of 0.53 g/100g (08-01), gluten content at 10.54 g/100g (38-10), Zeleny sedimentation value of 21 mL (56-60) and falling number at 432 s (56-81). Other ingredients for the production of bread such as sucrose powder, table salt and instant dry yeast were also procured from the local market.

2.2. SC-FOS (solid and liquid)

Solid and liquid samples of SC-FOS with DP 3-5 with 95% purity were supplied by M/s TATA Chemicals Limited Nellore, Andhra Pradesh, India. It is manufactured by Tata Chemicals Limited under the brand name FOSSENCE®. SC-FOS refers to oligomers of fructose having degree of polymerization 3-5 with a terminal glucose moiety where the fructose is linked by beta 2-1 linkage. FOSSENCE® has been produced through patented (Gote, Patil, Palamalai, Rengarajan, & Avalakki, 2014) biotransformation process from sucrose. It includes 40 ± 2% ketose (GF2), 47 ± 2 nystose (GF3) and 8 ± 2% of fructofuranosyl nystose (GF4).

2.3. Rheological characteristics

2.3.1. Empirical rheological characteristics

Wheat flour, wheat flour with 2.5 g/100g sucrose; 2.5g/100g SC-FOS and 5g/100g SC-FOS were studied to check the mixing profile using Brabender Farinograph (Duisburg, Germany). Control wheat flour, Farinograph water absorption was defined as the percent amount of water (14% flour moisture basis) required reaching a dough consistency of 500BU. The elastic profile was measured by extensograph (Brabender OHG, Duisburg, Germany) and the pasting profile by Micro-Visco-Amylograph (Brabender OHG, Duisburg, Germany) according to AACC methods (54-21, 54-10 and 22-10).

2.3.2. Fundamental rheological measurements

Rheometer (Anton Paar GmbH, Austria, Model: MCR 52) equipped with a parallel plate geometry (75 mm diameter, 1 mm gap) was used to

study the effect of sucrose and SC-FOS on the storage modulus (G') and loss modulus (G''). Dough was mixed at 30 °C until maximum development in a Hobart mixer (2 min at 58 rpm and 2 min at 112 rpm) at optimum water absorption (Farinograph water absorption) and placed between the parallel plates of the rheometer. Excess dough was trimmed and the exposed edge was coated with mineral oil to prevent drying and tested after resting for 10 min. Frequency sweep test from the range of 0.1–10 Hz and constant strain of 0.01% was performed within the linear viscoelastic range at 25 °C.

2.4. Bread making procedure and quality characteristics

Plain breads were prepared using straight dough method without the addition of preservatives (AACC 10-11, 2010). Flour (100 g), fresh yeast (2.0g), fat (1.0g), sucrose (0/2.5g), salt (1.0g) and water were mixed in a planetary mixer for 4 min at 61 rpm. Dough was fermented for 90 min at 30 °C and 75% relative humidity with a knock back, moulded after 25 min, proved for 55 min and finally baked for 25 min at 220 °C. Further sucrose was replaced by SC-FOS solid (s)/liquid (l) on weight basis at 2.5g/100g and on sweetness basis at 5g/100g for the next two samples. After baking, breads were cooled to room temperature for about 3–4 h. Breads were weighed and loaf volume was assessed by the rapeseed displacement method (Mallock and Cook, 1930). Volume per g of sample was calculated as specific volume. Crumb firmness of bread (25 mm slice) was measured using a texture analyser (TAHDI, Stable Micro Systems, Godalming, UK) by the standard method (AACC, 2010, Method 74-09).

The crumb color of the bread was measured in terms of lightness (L) and color (+a: red, – a: green, + b: yellow, – b: blue) using Hunter Color measuring system (Labscan XE, Hunter Associates Laboratory Inc, Reston, USA) with standard D 65 day light illuminate and 10° view angle against the standard made of barium sulphate (100 percent reflectance). The reflectance of bread crumb was measured in the wavelength range of 400–700 nm with an observer's angle of 10° in the sample holder. The average and standard deviation of three readings are used to calculate the values.

2.5. Sensory evaluation of breads

The sensory quality of breads was evaluated by qualitative descriptive analysis (QDA) by a group of male (10) and female (8) panelists of age between 25 and 55 years who are well versed with the quality parameters. Control and experimental breads were presented to the panelists by serving three-digit-randomly coded containers one after another for analyzing the sensory attributes of the product. The panelists evaluated the quality characteristics of breads by assigning scores in the score card for six sensory attributes on a nine point hedonic scale according to Meilgaard, Vance, and Thomas (1999); crust colour (1 = pale brown/dark brown; 9 = golden brown); crust shape (1 = flat; 9 = normal convex shape); crumb colour (1 = dull white/dark brown; 9 = creamish white); crumb grain (1 = coarse/closed and compact; 9 = fine and uniform); texture (1 = hard; 9 = soft) and mouthfeel (1 = dry/lumpy; 9 = clean mouthfeel with no residue). Panelists were served with water to rinse palate between the samples. The scores of all six quality attributes were combined to get the overall quality score.

2.6. Storage characteristics

Shelf life of breads prepared with and without sucrose and with SC-FOS (solid and liquid) was studied. For storage studies, breads were prepared using the standard procedures (AACC, 2010) along with preservatives, i.e. calcium propionate (0.3 g/100g) and glacial acetic acid (0.1 g/100g). Breads were cooled after baking, packed in polypropylene pouches of 250 µm and stored at ambient conditions of 27 °C and 65 g/100g relative humidity. Samples were analyzed for moisture, water activity, crumb firmness, crumb colour and overall acceptability every

alternate d till the appearance of visual growth of molds.

2.7. Scanning electron microscopy

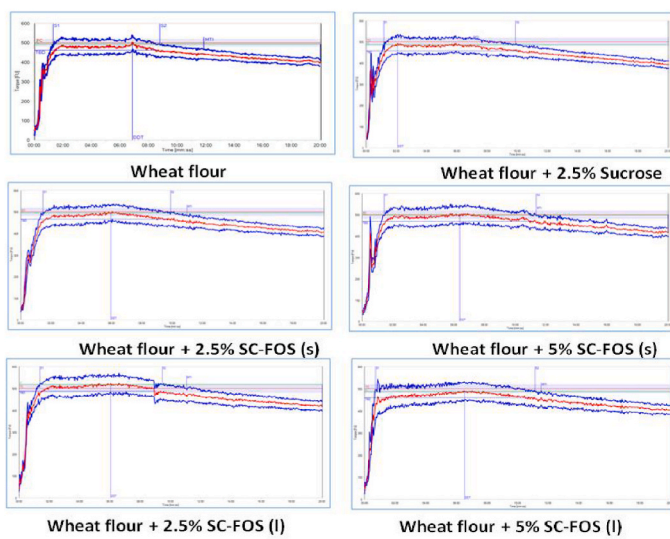
Scanning electron microscopic (SEM) studies for bread dough and bread crumb pieces were carried out using Leo scanning electron microscope Model 435 VP (Leo Electronic Systems, Cambridge, U.K.). The bread dough after mixing was sheeted and cut into pieces (size 10×10 mm) without damaging the structure and the same were freeze-dried using Heto freeze-dryer Model DW3 (Allerod, Denmark) at -53°C and 700 mm/Hg pressure. Bread crumb samples (20×20 mm) were defatted using hexane and then freeze-dried. Freeze-dried dough and bread samples were separately placed on the sample holder using double-sided scotch tape and was exposed to gold sputtering (2 min, 2 mbar). Further each sample was transferred to the microscope and

micrographs were observed at 15 kV and 9.75×10^{-5} torr vacuum.

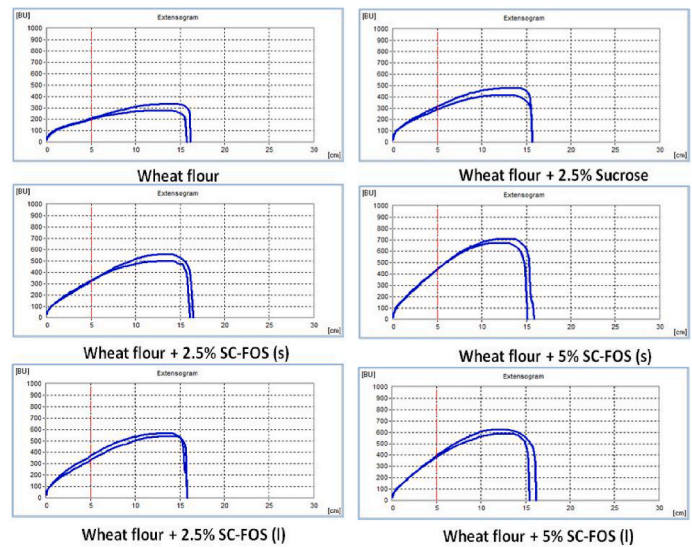
2.8. Powder X-ray diffraction

Powder X-ray Diffraction (PXRD) profiles were obtained from 20 to 30 mg of lightly ground sample was placed in a sample stage. X-ray diffraction data was collected on a PANalytical, "X"pert PRO (The Netherlands) diffractometer on a Cu source ($K\alpha - 1.5418$) powered by 40 kV and 30 mA with a proportional counter radiation detector. Data collection was done with step size 0.020° , 0.50 s per step over $4-40^\circ 2\theta$. Data was analyzed using Xpert Viewer software. PXRD profile data presented for the region where significant peaks were observed.

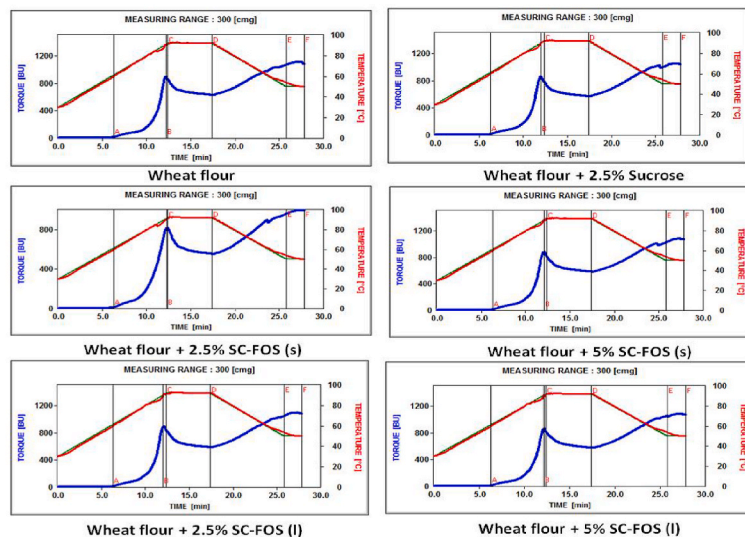
i: Farinograph characteristics



ii: Extensograph characteristics



iii. Pasting characteristics



iv. Storage and loss modulus

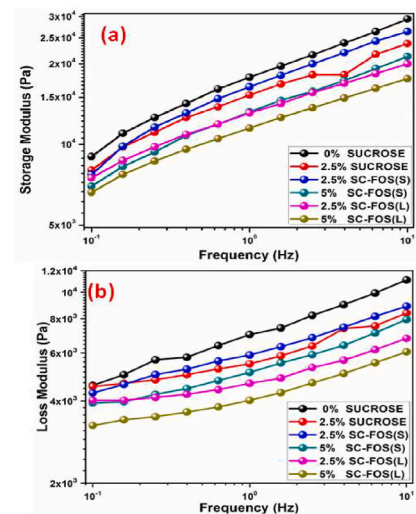


Fig. 1. Effect of sucrose and SC-FOS on the empirical and fundamental rheological characteristics (i) farinograph characteristics (ii) extensograph characteristics (iii) pasting characteristics (a) storage modulus (G') and (b) loss modulus (G'') of bread dough with frequency sweep mode.

2.9. Attenuated total reflectance (ATR) FTIR spectroscopy

FTIR spectra were collected using a Bruker Tensor II FTIR spectrophotometer (Bruker Optics, USA) over a wavelength range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} , equipped with OPUS operating system software. The semi-solid samples were covered on the surface in contact with attenuated total reflectance (ATR) on a multi-bounce plate of Zn-Se crystal at 25 °C. The sample scan time and the background scan time were 16 s, and the measurement time was 15 s. Structural analysis of the amide I band (1570–1720 cm^{-1}), amide III band (1200–1340 cm^{-1}) and OH stretching region (2500–4000 cm^{-1}) was conducted by using ORIGIN Software (Origin Lab Corporation, USA). A numerical deconvolution procedure with Gaussian functions was carried out to obtain individual contributions for distinctive bands. The absorbance intensities of bands around 1047, 1022 and 995 cm^{-1} were used to investigate the crystalline structures and molecular order degree of the starch.

2.10. Statistical analysis

The data related were expressed in terms of mean $\pm$ standard deviation. Statistical analysis of the data was done using Duncan's New Multiple Range Test using the software IBM SPSS Statistics (IBM Inc., New York, USA). A significance level of 5% was adopted for all comparisons.

3. Results and discussion

3.1. Empirical rheological characteristics

3.1.1. Mixing profile of the dough

Addition of sucrose or SC-FOS (solid or liquid) to the wheat flour dough influenced the Farinograph characteristics (Fig. 1). Addition of either sucrose or SC-FOS decreased the water absorption from 55.9 g/100g to 52 g/100g for 0g/100g and 5g/100g SC-FOS respectively. Wang, Rosell, & Barber (2002) and O'Brien, Mueller, Scannell, and Arendt (2003) reported that on addition of inulin there was a decrease in the water absorption. Sugars show a lubricating action and the degree of polymerization of oligosaccharides are known to influence the water absorption and formation of elastic networks (Rouillé, Della Valle, Lefebvre, Sliwinski, & Van Vliet, 2005; Zimeri & Kokini, 2002). The dough development time increased from 6.52 min to 7.04 min by the addition of SC-FOS indicating a delay in the gluten network formation. Dough stability which is measured as the difference in arrival time and departure time and which generally indicates the strength of flour increased from 8.11 min in control sample to 9.09 and 10.25 min by the addition of solid and liquid SC-FOS respectively indicating the strengthening effect of SC-FOS (Peressini & Sensidoni, 2009). According to Bojnanska, Tokar, and Vollmannova (2015), the dough development time which is the time to reach to 500 BU consistency, increased as a consequence of inulin addition. SC-FOS holds the water molecule to a greater extent and thereby reduces the availability of water for the gluten formation.

3.1.2. Elastic profile of the dough

The Extensograph characteristics of wheat flour (Fig. 1) shows an increase in resistance to extension from 308 to 392 BU and a decrease in extensibility from 164 to 154 BU indicating an increase in the stiffness of the dough. The increase in energy from 97 to 126 cm^2 indicate the strengthening of the dough by the addition of SC-FOS. Fiber addition have shortened and lowered the resistance to dough extension which is mainly due to the partial disruption of gluten network by the addition of insoluble and soluble cell wall materials (Collar, Santos, & Rosell, 2007). This has been in agreement with dough strength development observed during the mixing profile.

3.1.3. Pasting characteristics

Pasting profile includes pasting (gelatinization) temperature, peak viscosity, breakdown and setback of aqueous dispersions of wheat flour with sucrose and SC-FOS (Fig. 1). There is no difference in the gelatinization temperature by the addition of either sucrose or SC-FOS, while marginal increase in the peak viscosity from 820 to 880 BU was observed. Cold paste viscosity (CPV) was similar for wheat flour and wheat flour with 2.5g/100g SC-FOS samples (955 and 961 BU respectively) indicating no significant change when SC-FOS at 2.5g/100g was added to the wheat flour, whereas CPV slightly increased by the addition of 2.5g/100g sucrose from 955 to 997 BU which was related to the retrogradation of starch and staling of the product during storage. Formation of viscous paste is delayed during heating as the Oligosaccharides try to inhibit the swelling of starch granules, thereby lowering the available water for pasting of starch (Angioloni & Collar, 2009; Spies & Hoseney, 1982). Setback values decreased indicating that the addition of SC-FOS was effective at retarding the starch retrogradation and bread staling during storage. The reduction in setback values might have been due to the re-association of solubilized amylose and amylopectin polymers during the cooling phase (Katsuta, Miura, & Nishimura, 1992).

3.2. Fundamental rheological measurements

The storage modulus (G') and loss modulus (G'') as a function of the frequency for bread doughs with and without sucrose and with different levels of SC-FOS incorporation are illustrated in Fig. 1. The G' was higher in all the dough than the G'' indicating that all doughs were solid, elastic like behavior (Rubel, Perez, Manrique, & Genovese, 2015). As the increase in frequency, the magnitudes of G' and G'' were observed to be increased. The G' & G'' values of SC-FOS added dough were found to be lower as compared to control and sucrose added dough. The dough contained 2.5 g/100g sucrose and 2.5 g/100g SC-FOS (P) solid had nearly same value of G' & G'' , even though such observation in line with moisture, texture, sensory and PXRD results. Furthermore, the values decreased as the incorporation of SC-FOS were increased. Many studies have suggested the addition of soluble dietary fiber lowers the storage and loss modulus in comparison with ordinary or insoluble dietary fiber added dough (Rubel, Perez, Manrique, & Genovese, 2015). The decrease in G' and G'' values may be attributed to decrease in swelling power due to SC-FOS incorporation, which is good agreement with pasting characteristic results. It can be observed that, SC-FOS addition barely modified dough rheology. In particular, it is worth to note that storage modulus (G') decreased in the following order: 0 g/100g sucrose > 2.5 g/100g sucrose, 2.5 g/100g SC-FOS (s) > 2.5 g/100g SC-FOS (l) > 5 g/100g SC-FOS (s) > 5 g/100g SC-FOS (l). This implies that addition of 2.5% sucrose or 2.5 g/100g SC-FOS (P) had no significant changes in on dough rheology compared to 0% sucrose dough. On the other hand, addition of 2.5 g/100g, 5 g/100g SC-FOS (l) and 5 g/100g SC-FOS (s) alter the dough rheology. This alteration, may be attributed to the addition of SC-FOS with wheat flour which could disrupt the starch – protein matrix due to various forms of interactions.

3.3. Bread quality

Bread making is a process consisting of various processing conditions namely mixing, fermentation, proving, and baking; during the process of baking water gets evaporated, volume expands, starch gets gelatinized, protein gets denatured apart from the crust formation. Mixing helps in the formation of homogenous viscoelastic dough wherein changes in wheat protein namely breakage and formation of S-S bonds occur. (Haraszi, Larroque, Butow, Gale, & Bekes, 2008; Manu & Prasad Rao, 2008). The impact of sucrose and SC-FOS on bread properties is presented in Table 1. The control bread (0 g/100g sucrose) had 3.19 cc/g specific volume which increased on addition of either sucrose or SC-FOS (s/l). The heating of dough caused changes in the starch fraction (starch gelatinization), which dramatically increased the dough viscosity and

Table 1

Quality Characteristics of bread with sucrose and SC-FOS.

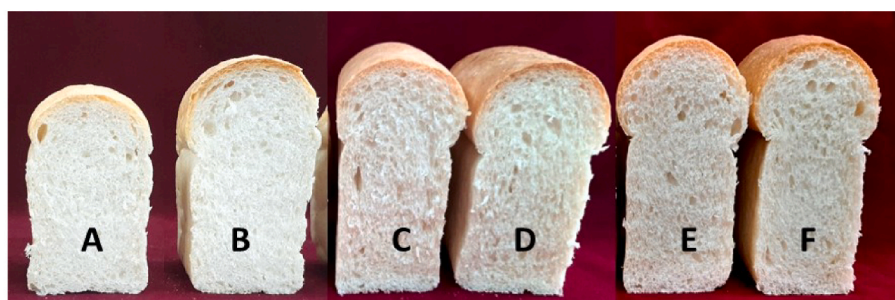
Sample	Specific volume* (cm ³ /g)	Crumb firmness** (g, force)	L*	Crust colour (9)	Crumb colour (9)	Texture (9)	Eating quality (9)
WF + 0 g/100g sucrose	3.19 ± 0.15 ^a	688 ± 5.2 ^d	65.26 ± 0.92 ^a	7.0 ^c	7.0 ^d	7.0 ^c	7.0 ^c
WF + 2.5 g/100g Sucrose	3.81 ± 0.12 ^b	498 ± 3.6 ^c	67.00 ± 0.56 ^b	8.0 ^b	7.5 ^c	8.0 ^b	8.0 ^b
WF + 2.5 g/100g SC-FOS (s)	3.81 ± 0.16 ^b	443 ± 2.9 ^b	65.61 ± 0.82 ^a	8.0 ^b	8.0 ^b	8.0 ^b	8.0 ^b
WF + 5 g/100g SC-FOS (s)	3.91 ± 0.12 ^c	366 ± 2.2 ^a	65.83 ± 0.65 ^a	8.5 ^a	8.5 ^a	8.5 ^a	8.5 ^a
WF + 2.5 g/100g SC-FOS (l)	3.81 ± 0.14 ^b	443 ± 4.2 ^b	65.82 ± 0.44 ^a	8.0 ^b	8.0 ^b	8.0 ^b	8.0 ^b
WF + 5 g/100g SC-FOS (l)	3.91 ± 0.11 ^c	366 ± 2.9 ^a	65.91 ± 0.31 ^a	8.5 ^a	8.5 ^a	8.5 ^a	8.5 ^a
SEM (±)	—	—	—	0.12	0.15	0.18	0.17

L- Lightness, (9)-Values in the parenthesis indicate maximum score for each attribute; Values with different letters in the same column are significantly different at P < 0.05; SEM - Standard error of mean at 102 degrees of freedom; Values are means ± standard deviation (*n = 3; ** = 6).

attained solid-like behavior (Peressini, Sensidoni, Pollini, & de Cindio, 1999). On addition of SC-FOS the volume increased and this could be due to the delay in gelatinization leading to higher dough expansion

during baking. The crumb firmness value decreased from 688 to 425 g and 366 g force by the addition of solid and liquid SC-FOS respectively indicating that addition of SC-FOS helped in retention of moisture and

a: Bread making quality



b: Quality characteristics of breads during storage

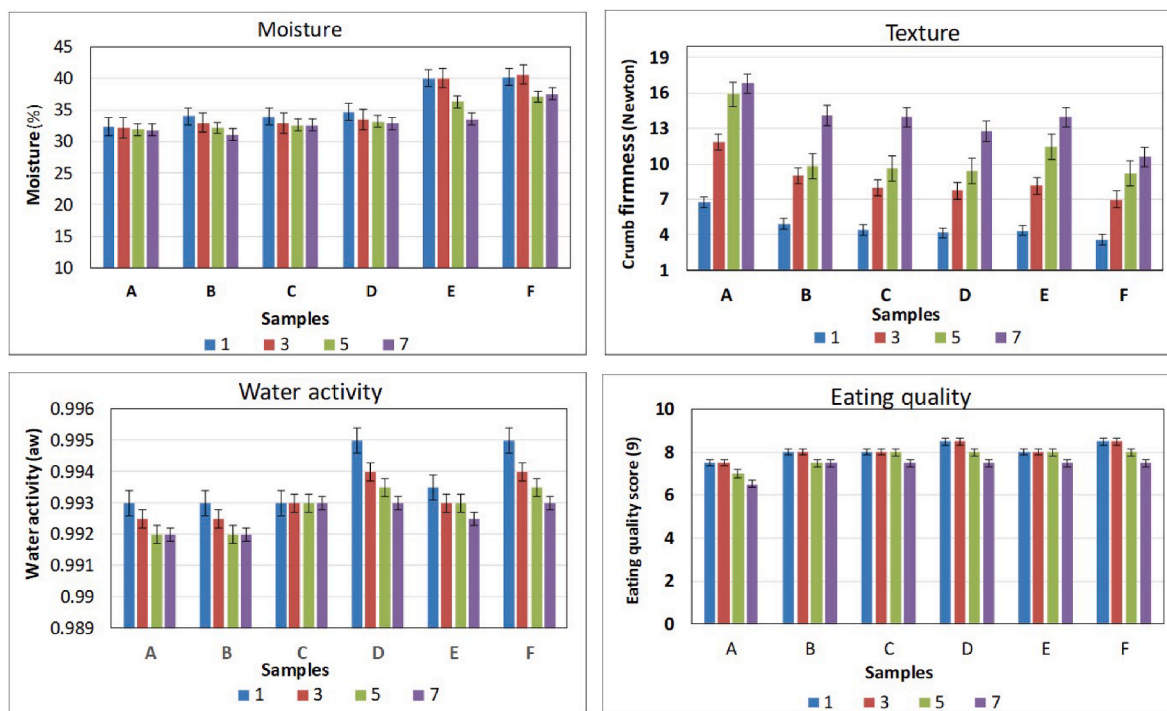


Fig. 2. Effect of sucrose and SC-FOS on the (a) bread making quality;

(b) quality characteristics of breads during 7-day storage period

A-0 g/100g Sucrose; B-2.5 g/100g Sucrose; C-2.5 g/100g SC-FOS (s); D- 5 g/100g SC-FOS (s); E-2.5 g/100g SC-FOS (l); F-2.5 g/100g SC-FOS (l).

gave the bread a softer texture. Retention of moisture by SC-FOS leads to lesser molecular mobility of water molecules which helps both intercepting gluten network and gelatinization.

Hunter colour values of bread samples revealed that bread without sucrose and SC-FOS had L value of 65.26 whereas L value of 2.5g/100g sucrose incorporated bread was 67 indicating slightly brighter crumb colour. The L value of 2.5g/100g and 5g/100g SC-FOS incorporated bread had no significant difference ($p < 0.5$) with 65.50 and 65.24 respectively compared to 2.5g/100g sucrose bread. The values of redness (a^*) and yellowness (b^*) did not differ significantly ($p < 0.5$) for all the breads. Several authors reported that the sugars and salt delay the starch gelatinization (Ghiasi, Hosene, & Varriano-Marston, 1982; Hoover & Senanayake, 1996; Spies & Hosene, 1982). Addition of SC-FOS marginally reduced the dough water absorption (Fig. 1), which is responsible for starch gelatinization during heating (Rolee & LeMeste, 1999; Wootton & Bamunuarachchi, 1979). Sensory evaluation of the breads showed that the bread without sucrose and SC-FOS showed lower scores because of light brown crust colour, creamish crumb colour, fine and dense cells, slightly hard texture with predominant salt taste because of the absence of sugar. Fig. 2 manifests the whole bread and cross section view of breads with and without sucrose and SC-FOS (solid and liquid). Bread without sucrose and SC-FOS had poor volume because of less gas cells with dense crumb. Rouille, Valle, Lefebvre, Sliwinski, & Vliet et al. (2005) observed that low molecular weight sugars reduced the crumb fineness which could be due to the preparation of doughs at constant hydration, thereby reflecting on the dough consistency and starch gelatinization. Bread with 2.5g/100g sucrose had typical golden brown crust colour, creamish white crumb colour and medium fine grain with medium soft texture. Incorporation of SC-FOS (solid or liquid) at 2.5g/100g had slightly brighter crumb colour, softer texture with mild sweetness compared to 2.5g/100g sucrose bread. While, 5g/100g SC-FOS incorporated bread had dark golden brown crust colour, slightly coarse grain and sweetish taste compared to 2.5g/100g SC-FOS bread. Rada-Mendoza, Garcia-Banos, Villamiel, and Olano (2004) reported that presence of higher levels of reducing sugars in inulin was responsible for the darker crust due to Maillard reaction during baking. According to the observation, incorporation of SC-FOS (s) at 2.5g/100g in replacement of 2.5g/100g of sucrose was found to be similar with respect to taste and sweetness. While with the incorporation of 5g/100g SC-FOS, slight increase in sweetness was observed along with softer texture when compared to 2.5g/100g replacement.

3.4. Storage quality of breads

Various parameters were evaluated for breads stored at ambient conditions by withdrawing the samples every alternate day for 7 d. During the storage period of 7 d, moisture content marginally decreased, water activity ranged between 0.92 and 0.95 and did not show any significant difference ($p < 0.05$) among the bread samples (Fig. 2). The crumb firmness values of all the breads have shown an increasing trend during storage. Breads without either sucrose or SC-FOS had a significant increase in crumb firmness values when compared to breads with either sucrose or SC-FOS (2.5 g/100g). Crumb firming was further lower at 5 g/100g level of SC-FOS. Similar results were observed when SC-FOS (l) was used in bread with 2.5 g/100g and 5 g/100g. The liquid SC-FOS in soluble form can have better mixing characteristics with higher molecular mobility in the bread matrix. Earlier studies have reported that there was an increase in moisture content of cookies with FOS which was hygroscopic in nature and acted as a humectant leading to softer cookie (De Soete, 2000). Similar properties for Oligofructose has been observed by Crittenden and Playne (2002), wherein they have reported that FOS functions as a humectant, thereby reducing the water activity and in turn would ensure the microbiological stability. Sensory evaluation of breads has shown that the eating quality scores reduced drastically for bread without sucrose or SC-FOS, whereas breads with 2.5g/100g sucrose and 2.5g/100g SC-FOS had similar scores. At 5g/100g SC-FOS the

textural quality of bread was comparatively better. Similar results were also observed when SC-FOS in liquid form was used, which shows even better softer crumb.

3.5. Scanning electron microscopy

3.5.1. Dough microstructure

The micrographs of bread dough with and without sucrose and with different levels of SC-FOS incorporation are depicted in Fig. 3. The effect of mechanical treatments like mixing, kneading, folding and impact of other ingredients such as water, sucrose and SC-FOS was observed as the association of starch granules and protein matrix. Micrograph of bread dough with 0 g/100g sucrose showed small and large starch granules discretely without any matrix. The 2.5g/100g sucrose incorporated bread dough showed starch granules enmeshed in protein matrix network. SEM evaluation of wheat flour doughs showed that the protein matrix formed a smooth, enveloping, veil-like network stretched over the starch granules (Khoo, Christianson, & Inglett, 1975; Evans, Volpe, & Zabik, 1977) where as in 2.5g/100g and 5g/100g SC-FOS(s) large and small starch granules were closely covered by small protein bodies and fiber network, in 5g/100g SC-FOS protein bodies were overlapped by large starch granules and found to be slightly discrete than 2.5g/100g SC-FOS dough with fibrous structures and in 2.5g/100g and 5g/100g liquid SC-FOS small and large starch granules were found to be bound together with clear film like structure without much discreteness.

3.5.2. Bread microstructure

The micrographs of freeze dried bread (Fig. 3) showed a continuous matrix of gelatinized starch fused and almost overlapping in all the samples, however, large starch granules, small starch granules were clearly observed in 0g/100g sucrose bread sample. Bread samples with 2.5g/100g and 5g/100g SC-FOS (solid) showed gelatinized starch granules submerged within protein and fiber matrix. Whereas, use of liquid SC-FOS at 2.5g/100g and 5g/100g levels on bread has shown a slight difference in microstructure compared to solid SC-FOS, where, the gelatinized large and small starch granules are enmeshed also discretely arranged within protein and fiber matrix. According to Zeng, Hu, Gao, Sun & Ma (2016), effect of fructooligosaccharide on wheat starch could be conducive for starch to form fine gel structures and could inhibit the retrogradation of wheat starch.

3.6. Powder X-ray diffraction

Starch exists in semi-crystalline granules in native form. Starch form two type of crystal lattice A-type and B-type Polymorph. These polymorphs can be distinguishing peaks and are less intense because of gelatinization (Fig. 4). The presence of peaks at 17° , 22° and 24° 2θ indicates the formation of B-type polymorph of starch in the bread. All the batches of bread show an increase in crystallinity towards the B-type polymorph over 7 d. The bread with 0g/100g and 2.5g/100g sucrose showed the highest crystallinity. Crystallinity decreased as the percent composition of SC-FOS increased. The crystallinity of 2.5g/100g sucrose and 2.5g/100g SC-FOS were almost similar as most of the sucrose and SC-FOS in batches is used up for the activation of yeast. Hence very less is available to affect the crystallization of starch in the matrix. However, the crystallization of starch significantly decreased when SC-FOS was incorporated at 5g/100g and above as evident from Fig. 4. This observation is in line with softness of bread found in sensory analysis. SC-FOS holds the moisture better and modulates moisture migration; hence decrease of starch crystallization in bread leads to softer matrix.

3.7. Attenuated total reflectance (ATR) FTIR spectroscopy

The 995/1022 ratio showed a decreasing trend with respect to storage time/day (Table 2). This trend indicates the retention of hydrated starch structures when compared with blank and sucrose added

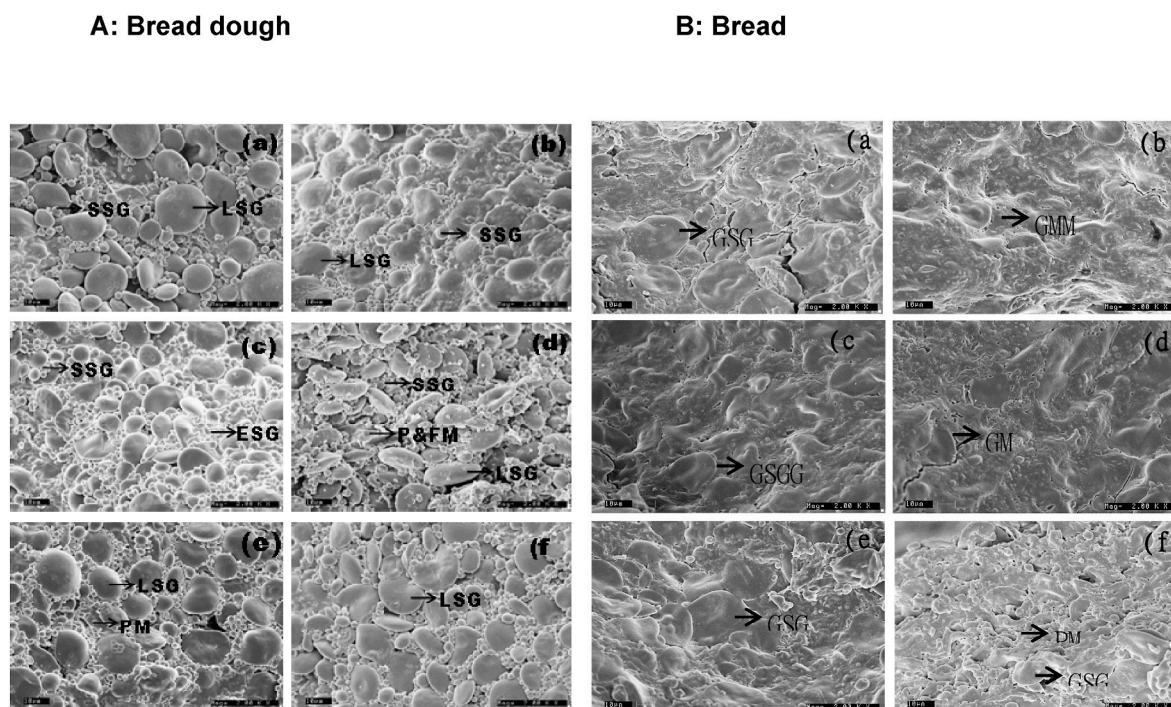


Fig. 3. Scanning Micrographs of bread dough and bread
SSG- Small starch granules; LSG-Large starch granules; PM-Protein matrix;
GSG-Gelatinized starch granule; GM- Gluten matrix; PM-Protein matrix
-0 g/100g Sucrose; b-2.5 g/100g Sucrose; c-2.5 g/100g SC-FOS (s); d- 5 g/100g SC-FOS (s); e-2.5 g/100g SC-FOS (l); f-2.5 g/100g SC-FOS (l).

bread. These results agree with the changes in moisture content & crumb firmness values during the storage (Fig. 4). The ratio of 995/1022 has been linked to the ratio between the hydration and amorphous extent of starch granules (Table 2). Consequently, the 1047/1022 ratio reflects the sensitive to the ordered/crystalline and to the amorphous starch granules. However, this ratio confirms the domination of crystalline or amorphous status of starch. The results in Table 2 suggested that the I (1047)/I (1022) ratio increased for blank bread with respect to storage time. On the other hand, SC-FOS added bread displayed a reverse trend which contains reduced quantity of crystalline starch structure. Moreover, it is noteworthy to mention that the presence of SC-FOS might act as a physical barrier to prevent associations between starch granules during storage. These results imply that presence of SC-FOS impede the starch retrogradation of amylose and amylopectin. This correlation is supported by the pasting characteristic results displayed in previous section. The band ratio 995/1022 for SC-FOS added bread were higher than that of with and without sucrose added breads, indicating that the moisture content of SC-FOS added bread in continuous phase was higher than that of with and without sucrose added bread with respect to storage time/day. Furthermore, compared to both with and without sucrose added breads, the absorbance ratio of SC-FOS added breads held for 5 and 7 d did not show a sharp fall. The reduced hydrated structures of starch might be linked to the increased firmness of the bread crumb is in good agreement with textural results. Upon increasing the storage time/day, the bread without the SC-FOS reflected a poor retention of amorphous/hydrated starch structure, which may be due to the formation of highly crystalline starch phases during the storage. This correlation is supported by the PXRD results demonstrated in Fig. 4 suggesting that SC-FOS delayed the retrogradation of starch. These observations also suggested that SC-FOS added bread was more favoring to hold the water molecules in the matrix. For clear understanding, FOS are highly hygroscopic compounds and their water holding capacity is major to that of sucrose and equal to that of sorbitol (Morris & Morris, 2012). Although previous studies demonstrated that FOS act as a humectant, which greatly suppress the drying of bread and other bakery

products (Morris & Morris, 2012). It is scientifically very interesting for understanding the role of FOS in bread. Unlike other traditional humectant agent and sugar replacer FOS acts as a multifunctional ingredient/additive, which serve as a numerous functional/physicochemical property in the bread matrix. It has been understood that as per nutritional point of view FOS is classified as a dietary fibers and prebiotics (J Slavin, 2013; Sabater-Molina, Larqué, Torrella, & Zamora, 2009). Apart from this, reduction in glycemic index with addition of optimum amount of FOS in biscuits (Padmaishwarya, Prabhasankar, & P, 2013). Most probably, SC-FOS forms a thin layer or partially encapsulate the starch/protein matrix. This behavior moderately hinders the diffusion of water molecules from the starch/protein matrix during the storage. As SC-FOS hold moisture better and restrict water mobility in matrix, this effect may be involved in our SC-FOS added bread for lesser retrogradation of bread.

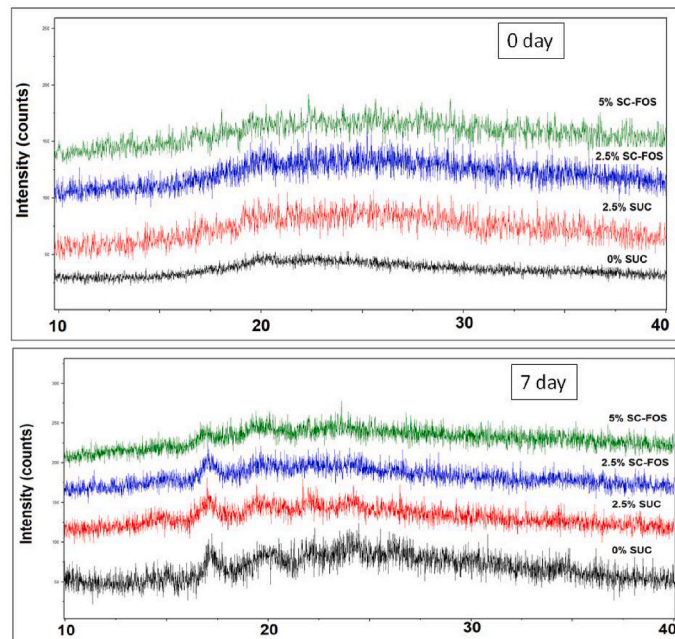
3.8. Relationship between empirical and analytical data

As observed by the studies conducted, starch crystallization has decreased by the addition of SC-FOS as reflected in FTIR. The dough rheological characteristics such as dough stability (8.11–9.09 min) and dough development time (5.4–7.04 min) increased with increase in peak viscosity and cold paste viscosity as supported by the retention of moisture content throughout seven days of storage. On the other hand, liquid SC-FOS increased the hydration of starch as reflected by the amylograph data as well as moisture retention, crumb firmness values and smooth surface of bread. Dough rheological characteristics show the decrease in water absorption and increase in dough stability thereby strengthening the gluten network which is because of the addition of SC-FOS.

4. Conclusion

Addition of SC-FOS has shown better bread quality characteristics with respect to moisture retention and softness till the end of storage

(i) PXRD profile



(ii) FTIR spectra

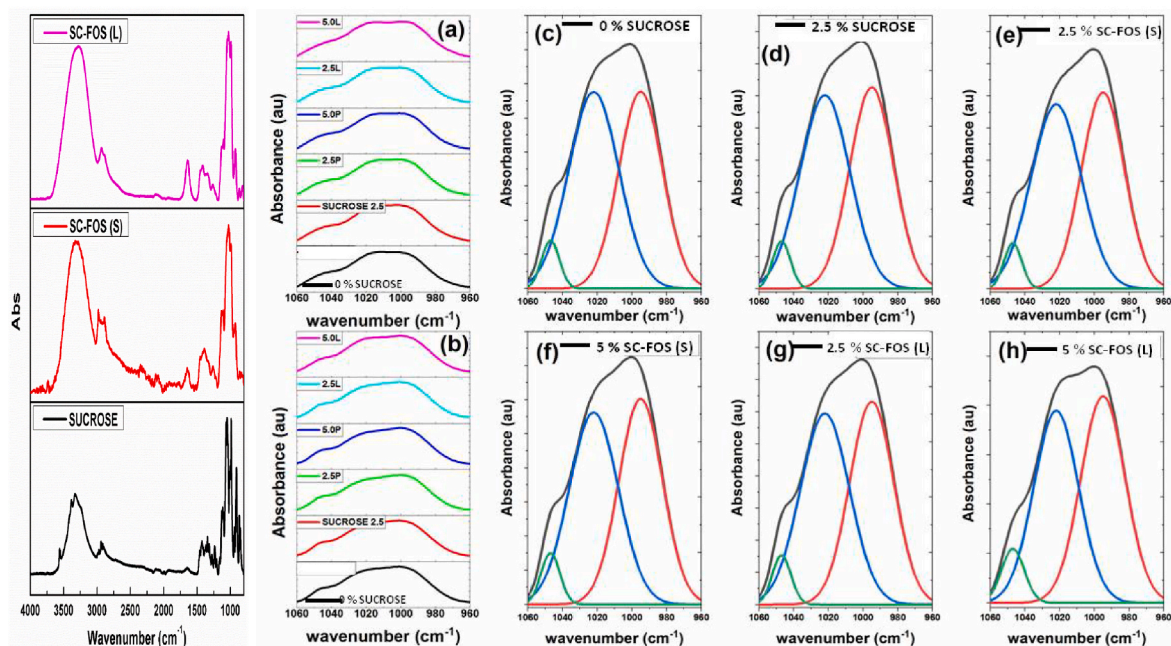


Fig. 4. (i)PXRD profile of bread with sucrose and SC-FOS during storage
(ii) IR spectra of ingredients and DAY 1 bread with sucrose and S
C-FOS (a)
Base line corrected IR spectra. (b) Cumulative fitted IR spectra. (c-h) Peak fitted IR spectra.

period. SC-FOS (solid) at 5g/100g and SC-FOS (liquid) at 2.5g/100g on bread has shown better results when replaced with sucrose. In product like bread where moisture content is higher, SC-FOS can successfully work as good humectant and yield softer product over shelf of 7 d. SC-FOS has shown better control mobility of water in bread matrix, which ultimately leads to lesser crystallization of starch and softer bread. These positive attributes observed in SC-FOS added bread has been studied from structural, micro structural and supra molecular

perspective and able to demonstrate mechanistic insights through the study. Thereby, addition of SC-FOS has given a promising result with better quality and also excellent sugar replacer in bread. Hence, SC-FOS can therefore act as an alternative to sugar and a boon to diabetic population.

Table 2

Intensity ratio 995/1022 reflecting the ratio between hydrated and amorphous structures and Intensity ratio 1047/1022 reflecting the ratio between ordered and amorphous structures in bread during storage.

IR ratio	Sample	DAY 1	DAY 5	DAY 7
995/1022	0 g/100g Sucrose	1.02 ± 0.003 ^a	0.72 ± 0.006 ^a	0.66 ± 0.007 ^a
	2.5 g/100g Sucrose	1.05 ± 0.004 ^b	0.75 ± 0.017 ^b	0.75 ± 0.008 ^b
	2.5 g/100g SC-FOS (s)	1.07 ± 0.005 ^c	0.79 ± 0.007 ^c	0.74 ± 0.007 ^b
	5.0 g/100g SC-FOS (s)	1.08 ± 0.007 ^d	0.84 ± 0.003 ^e	0.83 ± 0.017 ^c
	2.5 g/100g SC-FOS (l)	1.08 ± 0.013 ^{cd}	0.82 ± 0.004 ^d	0.82 ± 0.004 ^c
	5.0 g/100g SC-FOS (l)	1.16 ± 0.003 ^e	0.93 ± 0.009 ^f	0.87 ± 0.002 ^d
1047/1022	0 g/100g Sucrose	0.24 ± 0.003 ^a	0.25 ± 0.005 ^c	0.33 ± 0.010 ^e
	2.5 g/100g Sucrose	0.25 ± 0.004 ^a	0.22 ± 0.014 ^a	0.26 ± 0.006 ^d
	2.5 g/100g SC-FOS (s)	0.25 ± 0.002 ^a	0.23 ± 0.001 ^{ab}	0.17 ± 0.002 ^a
	5.0 g/100g SC-FOS (s)	0.27 ± 0.006 ^b	0.22 ± 0.011 ^a	0.19 ± 0.003 ^b
	2.5 g/100g SC-FOS (l)	0.26 ± 0.019 ^{ab}	0.24 ± 0.006 ^b	0.27 ± 0.008 ^d
	5.0 g/100g SC-FOS (l)	0.28 ± 0.009 ^b	0.25 ± 0.004 ^c	0.20 ± 0.003 ^c

Values with different letters in the same column are significantly different at $P < 0.05$.

Values are means ± standard deviation (n = 3).

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CRedit authorship contribution statement

M.L. Sudha: Investigation, Visualization, Data curation, Interpretation of the data, Writing – original draft. **C. Soumya:** Methodology, Investigation, Data curation, Formal analysis, Writing – review & editing. **M. Saravanan:** Methodology, Investigation, Formal analysis, Writing – review & editing. **P. Madhushree:** Methodology, and, Formal analysis. **Jiviyot Singh:** Methodology, and, Formal analysis, Investigation. **Saikat Roy:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **P. Prabhasankar:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

AACC. (2010). *Approved methods of the American association of cereal chemists* (11th ed.). St. Paul, MN: AACC International.

- Angioloni, A., & Collar, C. (2009). Bread crumb quality assessment: A plural physical approach. *European Food Research and Technology*, 229, 21–30.
- Bojnanska, T., Tokar, M., & Vollmannova, A. (2015). Rheological parameters of dough with inulin addition and its effect on bread quality in Proc. Conference Series. *Journal of Physics*, 602.
- Collar, C., Santos, E., & Rosell, C. M. (2007). Assessment of the rheological profile of fibre-enriched bread doughs by response surface methodology. *Journal of Food Engineering*, 78, 820–826.
- Crittenden, R., & Playne, M. (2002). Purification of food-grade oligosaccharides using immobilised cells of *Zymomonas mobilis*. *Applied Microbiology and Biotechnology*, 58, 297–302.
- De Soete, J. (2000). Prebiotic ingredients in chewing gum—the use of inulin and oligofructose. *The Manufacturing Confectioner*, 80, 67–69.
- Evans, L. G., Volpe, T., & Zabik, M. E. (1977). Ultrastructure of bread dough with yeast single cell protein and/or emulsifier. *Journal of Food Science*, 42, 70–74.
- Ghiasi, K., Hoseney, R. C., & Varriano-Marston, E. (1982). Effects of flour components and dough ingredients on starch gelatinization. *Cereal Chemistry*, 60, 58–61.
- Gote, M., Patil, G., Palamalai, M., Rengarajan, S., & Avalakki, U. K. (2014). *Process for production of fructooligosaccharides*. US Patent No 8 (Vol. 871), 476.
- Haraszi, R., Larroque, O. R., Butow, B. J., Gale, K. R., & Bekes, F. (2008). Differential mixing action effects on functional properties and polymeric protein size distribution of wheat dough. *Journal of Cereal Science*, 47, 41–51.
- Hess, J. R., Birkett, A. M., Thomas, W., & Slavin, J. L. (2011). Effects of short-chain fructooligosaccharides on satiety responses in healthy men and women. *Appetite*, 56, 128–134.
- Hirayama, M., Sumi, N., & Hidaka, H. (1989). Purification and properties of fructooligosaccharides producing beta-fructofuranosidase from *Aspergillus Niger* ATCC 20611. *Agricultural & Biological Chemistry*, 53, 667–673.
- Hoover, R., & Senanayake, N. (1996). Effect of sugars on the thermal and retrogradation properties of oat starches. *Journal of Food Biochemistry*, 20, 65–83.
- Katsuta, K., Miura, M., & Nishimura, A. (1992). Kinetic treatment for rheological properties and effects of saccharides on retrogradation of rice starch gels. *Food Hydrocolloids*, 6, 187–198.
- Kho, C., Christianson, D. D., & Inglett, G. E. (1975). Scanning and transmission microscopy of dough and bread. *Baker's Digest*, 49, 24–26.
- Malloock, J. G., & Cook, W. K. (1930). A volume measuring apparatus for small loaves. *Cereal Chemistry*, 7, 307–310.
- Manu, B. T., & Prasad Rao, U. J. S. (2008). Influence of size distribution of proteins, thiol and disulfide content in whole wheat flour on rheological and chapati texture of Indian wheat varieties. *Food Chemistry*, 110, 88–95.
- Meilgaard, M., Vance Civille, G., & Thomas Carr, B. (1999). Controls for Test Room, Product, and Panel. In Morten. C. Meilgaard (Ed.), *Sensory evaluation techniques* (3rd ed.). CRC Press.
- Molis, C., Flourie, B., Ouarne, F., Gailing, M. F., Lartigue, S., Guibert, A., et al. (1996). Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. *The American Journal of Clinical Nutrition*, 64, 324–328.
- Morris, C., & Morris, G. A. (2012). The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: A review. *Food Chemistry*, 133, 237–248.
- O'Brien, C. M., Mueller, A., Scannell, A. G. M., & Arendt, E. K. (2003). Evaluation of the effects of fat replacers on the quality of wheat bread. *Journal of Food Engineering*, 56, 265–267.
- Padma Ishwarya, S., Prabhasankar, P., & P. (2013). Fructooligosaccharide – retention during baking and its influence on biscuit quality. *Food Bioscience*, 4, 68–80.
- Peressini, D., & Sensidoni, A. (2009). Effect of soluble dietary fibre addition on rheological and bread making properties of wheat doughs. *Journal of Cereal Science*, 49, 190–201.
- Peressini, D., Sensidoni, A., Pollini, C. M., & de Cindio, B. (1999). Improvement of wheat fresh pasta-making quality. Influence of sodium chloride on dough rheological properties. *Italian Food and Beverage Technology*, 17, 20–23.
- Priyali, S., Thomas, M. S. W., Alexandra, L. J., Adish, E., Janice, C., Andreea, Z., et al. (2021). Acute glycemic and insulin response of Fossece™ alone, or when substituted or added to a carbohydrate challenge: A three-phase, acute, randomized, cross-over, double blind clinical trial. *Heliyon*, 7, Article e06805.
- Rada-Mendoza, M., Garcia-Banos, J. L., Villamiel, M., & Olano, A. (2004). Study on nonenzymatic browning in cookies, crackers and breakfast cereals by maltulose and furosine determination. *Journal of Cereal Science*, 39, 167–173.
- Rolee, A., & LeMeste, M. (1999). Effect of moisture content on thermomechanical behaviour of concentrated wheat starch-water preparations. *Cereal Chemistry*, 76, 452–458.
- Rouillé, J., Della Valle, G., Lefebvre, J., Sliwinski, E., & Van Vliet, T. (2005). Shear and extensional properties of bread doughs affected by their minor components. *Journal of Cereal Science*, 42, 45–57.
- Rubel, I. A., Perez, E. E., Manrique, G. D., & Genovese, D. B. (2015). Fibre enrichment of wheat bread with Jerusalem artichoke inulin: Effect on dough rheology and bread quality. *Food Structure*, 3, 21–29.
- Sabater-Molina, M., Larqué, E., Torrella, F., & Zamora, S. (2009). Dietary fructooligosaccharides and potential benefits on health. *Journal of Physiology & Biochemistry*, 65, 315–328.
- Slavin, J. (2013). Fiber and prebiotics: Mechanisms and health benefits. *Nutrients*, 5(4), 1417–1435.
- Spies, R. D., & Hoseney, R. C. (1982). Effect of sugars on starch gelatinization. *Cereal Chemistry*, 59, 128–131.
- Storey, D., Lee, A., Bornet, F., & Brouns, F. (2007). Gastrointestinal tolerance of erythritol and xylitol ingested in a liquid. *European Journal of Clinical Nutrition*, 61, 49–354.

- Vigneshwari, K., & Manonmani, K. (2020). Effect of sugar replacer (stevia and maltitol) on quality characteristics of bread. *Asian Journal of Dairy and Food Research*, 39, 159–163.
- Wang, J., Rosell, C. M., & de Barber, C. B. (2002). Effect of the addition of different fibres on wheat dough performances and bread quality. *Food Chemistry*, 79, 221–226.
- Wootton, M., & Bamunuarachchi, A. (1979). Application of differential scanning calorimetry to starch gelatinization. II. Effect of heating rate and moisture level. *Starch*, 31, 262–264.
- Yadav, D. N., Patki, P. E., Sharma, G. K., & Bawa, A. S. (2009). Role of ingredients and processing variables on the quality retention in frozen bread doughs: A review. *Journal of Food Science & Technology*, 46, 12–20.
- Zeng, J., Hu, Y., Gao, H., Sun, J., & Ma, H. (2016). Fructooligosaccharides impact on the hydration and retro-gradation of wheat starch and gel. *International Journal of Food Properties*, 19, 2682–2692.
- Zimeri, J. E., & Kokini, J. L. (2002). The effect of moisture content on the crystallinity and glass transition temperature of inulin. *Carbohydrate Polymers*, 48, 299–304.