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# Original article

# Effects of fat replacement and fibre addition on the texture, sensory acceptance and structure of sucrose-free chocolate

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**Summary** Dietary fibre has been employed as a sucrose and fat replacement in chocolates and can influence the physical and sensory characteristics of the resulting product. Formulations of sucrose-free chocolates were developed with the addition of inulin and  $\beta$ -glucan concentrate as partial substitutes for cocoa butter using a mixture design. The effects of the combinations of the three ingredients provided for the design on the texture, microstructure and sensory acceptance of the chocolates were investigated. The substitution of cocoa butter for inulin or  $\beta$ -glucan concentrate decreased the hardness of the chocolates. It was possible to replace 10 g of cocoa butter in a 100-g control formulation with inulin and still maintain good acceptance, while this same substitution with  $\beta$ -glucan resulted in less acceptable chocolate, with a mean score of 6.4 on a scale from 0 to 10. Scanning electron microscopy (SEM) was used to investigate the effects of fibre addition by observing the developed microstructure.

**Keywords** Cocoa butter, inulin, scanning electron microscopy, sensory acceptance,  $\beta$ -glucan.

#### Introduction

Along with visual appearance, flavour and aroma, texture is a primary attribute that defines food sensory quality and flavour. Texture evaluation is a complex and dynamic process that includes the visual perception of the product surface, the product behaviour upon initial handling and the integration of the oral sensations experienced during chewing and swallowing, which are compiled by the human brain in one unique sensation of texture (Costell & Durán, 2005).

The sensory attributes of chocolate are strongly dependent on the proportions and distribution of the solids on the cocoa butter capacity to form a stable crystalline structure. (Rousseau, 2006). As the ingredients involved have diameters smaller than 100  $\mu$ m, the microstructure analysis can be a useful tool for evaluating the texture and sensory characteristics of chocolate (Aguilera, 2005).

Hardness is one of texture attributes that stems from the established microstructure and is therefore influenced not only by the composition but also by the processing conditions, especially tempering and the

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crystallised lipid phase polymorphism that tempering induces. Proper tempering promotes hardness, shine and durability against physical and thermal damage (Afoakwa *et al.*, 2008; Lee *et al.*, 2009).

Due to this interdependence, the development of chocolate formulations that appeal to consumers seeking reduced sugar, fat and calories while maintaining quality and flavour is a challenge.

Sucrose-free chocolates can be successfully produced. Researchers have characterised their rheological behaviour and texture (Vissotto *et al.*, 2005; Shah *et al.*, 2010), investigated the influence of different body agents on the rheological and sensory characteristics of chocolate low in sucrose and calories (Gomes *et al.*, 2007), assessed its sensory acceptance (Melo *et al.*, 2009) and optimised formulations due to their rheological, microstructure and physical quality characteristics (Aidoo *et al.*, 2014).

Inulin has a prebiotic effect because it is fermented by bifidobacteria in the colon (BeMiller & Huber, 2007). It is a dietary fibre and may be substituted for fats and sugars without affecting the texture of foods, leading to products with a more balanced nutritional composition (Frank, 2002). Its use as a body agent in chocolates is efficient, as observed by Farzanmehr & Abbasi (2009), who studied the effects of inulin, polydextrose and maltodextrin on physicochemical, textural and sensory properties of milk chocolate. The authors kept good texture and sensory acceptance with complete substitution of sucrose content by inulin, reaching 41.8 g inulin/100 g chocolate; the higher scores were related to the formulations that contained from 14 to 32 g inulin/100 g chocolate. Shah *et al.* (2010) analysed the sensory and physical-chemical properties of dietetic chocolates with 16 g inulin/100 g, and Gomes *et al.* (2007) performed the same tests with 32 g inulin/100 g with good results.

 $\beta$ -glucan efficaciously reduces the level of cholesterol, contributing to more stable blood glucose and reducing the risk of colon cancer (Lim *et al.*, 2010). Because it is a high-molecular weight polymer, it is used in the food industry for its high capacity to retain water and for its thickening, jellifying and emulsifying properties. Lee *et al.* (2009) used 30 g/ 100 g  $\beta$ -glucan concentrate in chocolates as a partial substitute for cocoa butter and found that at over 10 g/100 g substitution, the viscosity of the chocolates increased to unacceptable levels and yielded softer products. However, the samples were not submitted to sensory evaluation.

Considering the Brazilian consumer preference for milk chocolate kind, skim milk powder was used in this study, conserving also its positive influence in taste development. However, the milk chocolate nomenclature may require legal parameters that are different in other places, as Europe and United States, where the legislation demands that minimal concentrations of milk fat must to be added too. So even though the milk solids are present, the products cannot be classified as milk chocolate in some countries.

In a previous work (Rezende *et al.*, 2014), we study the effect of  $\beta$ -glucan concentrate and inulin as partial substitutes for cocoa butter in rheological and sensory properties (descriptive analysis) of sucrose-free chocolate, using a constrained mixture design. The objective of the present work was to complement the characterisation of those products through the analysis of texture, sensory acceptance and microstructure.

## Materials and methods

## Materials

The formulations were produced with cocoa liquor (Barry Callebaut, Extrema, Brazil), cocoa butter (Barry Callebaut, Ilhéus, Brazil), skimmed milk powder (Tangará Foods, Vila Velha, Brazil), extra fine sugar (Glaçúcar, União, Piedade, Brazil), extra fine sugar (Glaçúcar, União, Piedade, Brazil), maltitol (ECIL Ingredientes, São Paulo, Brazil), stevia highintensity edulcorant (Enliten<sup>™</sup>, Corn Products, Mogi Guaçu, Brazil), soy lecithin (Solae do Brasil Ltda, Barueri, Brazil), polyglycerolpolyricinoleate – PGPR (Danisco Brasil Ltda, Pirapozinho, Brazil), inulin (Orafti<sup>®</sup>GR; Beneo, Oreye, Belgium) and  $\beta$ -glucan concentrate (Barley Balance<sup>TM</sup>; PolyCell Technologies, Crookston, MN, USA), with 38 g/100 g dietary fibre, being 27 g/100 g  $\beta$ -glucan and 11 g/100 g other insoluble fibre.

## Experimental design

A simplex-centroid design was used with a three-component mixture, with lower and upper limit restrictions and central points (Statsoft, 2010). The studied variables were as follows: inulin (0-50 g/100 g),  $\beta$ -glucan concentrate (0-50 g/100 g) and cocoa butter (50-100 g/100 g). Table 1 shows the ternary mixture component ratios in real concentrations and as pseudocomponents.

In preliminary tests, the conching stage of the chocolate production revealed that the cocoa butter reduction should be limited to 50 g/100 g in order to allow proper mixing of all the ingredients. Thus, the lower limit restriction of cocoa butter was 50 g/100 g. The higher level was maintained as 100 g/100 g to represent a formulation without fibre addition. Therefore, the two other components were restricted to upper limits of 50 g/100 g and lower limits of 0 g/100 g, where the lower limit was used to demonstrate the effect of only one type of fibre substitution.

The design consisted of eight formulations, one (BG3.2 + IN3.2') being a repetition of the central point (BG3.2 + IN3.2), to verify the standardisation of the process by calculation of the experimental error. A standard formulation produced with sucrose and without fibre addition was included in the experiment as an external control and for comparison with the dietetic chocolate formulations.

Table 2 gives the chocolate formulations with inulin,  $\beta$ -glucan and cocoa butter combined with the other ingredients according to the experimental design. In all formulations, the concentration of cocoa liquor, milk powder, lecithin, maltitol + edulcorant and PGPR was kept constant. The sucrose used in traditional chocolate was replaced with the same concentration (34 g/100 g) of a mixture of maltitol with 0.04 g/100 g (of the total formulation weight) of highintensity edulcorant.

#### Chocolate production

Cocoa liquor was mixed with the powder ingredients (skim milk powder, sucrose, maltitol + edulcorant, inulin and  $\beta$ -glucan concentrate) and with 2/3 of the corresponding cocoa butter in a planetary mixer (PPA-P; Inco S. A., Avaré, Brazil) at 40 °C for 10 min.

Total Fat

29.2 19.2 19.2 24.0 24.0 18.0

22.0

22.0

29.2

**Table 1** Experimental design for ternary mixtures of inulin,  $\beta$ -glucan concentrate and cocoa butter in real proportions and in pseudocomponents

Formulation*	Component proportions in the ternary mixture							
	Real conce	ntrations (%)		Pseudocomponents <sup>†</sup>				
	Inulin	β-glucan concentrate	Cocoa butter	Inulin (X <sub>1</sub> )	β-glucan concentrate ( <i>X</i> <sub>2</sub> )	Cocoa butter ( <i>X</i> <sub>3</sub> )		
СВ	0.0	0.0	100.0	0.000	0.000	1.000		
IN10	50.0	0.0	50.0	1.000	0.000	0.000		
BG10	0.0	50.0	50.0	0.000	1.000	0.000		
BG5	0.0	25.0	75.0	0.000	0.500	0.500		
IN5	25.0	0.0	75.0	0.500	0.000	0.500		
BG5 + IN5	25.0	25.0	50.0	0.500	0.500	0.000		
BG3.2 + IN3.2	16.6	16.6	66.8	0.333	0.333	0.333		
BG3.2 + IN3.2'	16.6	16.6	66.8	0.333	0.333	0.333		

\*CB, cocoa butter; IN, inulin; BG, β-glucan. The numbers in the samples codes represent the concentration of the ingredient in the chocolate formulation, as showed in Table 2.

<sup>†</sup>Calculated with equations:  $X_1 = (C_{inulin} - 0.00)/0.50 X_2 = (C_{\beta-glucan} - 0.00)/0.50 X_3 = (C_{CB} - 0.50)/00.5.$ 

Formulation*	Cocoa liquor	Skim milk	Lecithin	PGPR	Sucrose	Maltitol + Edulcorant	Cocoa butter	β-glucan concentrate	Inulin
СВ	30	15.5	0.3	0.2	0	34	20	0	0
IN10	30	15.5	0.3	0.2	0	34	10	0	10
BG10	30	15.5	0.3	0.2	0	34	10	10	0
BG5	30	15.5	0.3	0.2	0	34	15	5	0
IN5	30	15.5	0.3	0.2	0	34	15	0	5
BG5 + IN5	30	15.5	0.3	0.2	0	34	10	5	5
BG3.2 + IN3.2	30	15.5	0.3	0.2	0	34	13.6	3.2	3.2
BG3.2 + IN3.2'	30	15.5	0.3	0.2	0	34	13.6	3.2	3.2
Control	30	15.5	0.3	0.2	34	0	20	0	0

 Table 2
 Proportions (g/100 g) of ingredients in chocolate formulations

\*CB, cocoa butter; IN, inulin; BG, β-glucan; PGPR, polyglycerolpolyricinoleate. The numbers in the samples codes represent the concentration of the ingredient in the chocolate formulation.

<sup>†</sup>Refers to the cocoa butter added in formulation plus the amount naturally contained in cocoa liquor.

A three-cylinder pilot mill (Draiswerke GmbH, Mannhein, Germany) was used to refine the mass to a 17- to 23-µm particle size, determined with a digital micrometre (Mitutoyo Sul Americana Ltda, Suzano, Brazil).

Conching was performed in a planetary mixer at 60 °C for 14 h. The emulsifiers and the remaining cocoa butter (1/3) were incorporated at the beginning of this stage.

The temper procedure was made with the traditional method of temperature thermal shock on a marble surface at 18 °C. The initial mass temperature was 50 °C, and the mass was frequently revolved with spatulas for fast heat dissipation until the temperature fell to 27 °C.

The process efficiency was assessed with a tempermeter (E5; Sollich, Bad Salzuflen, Germany), and this equipment classifies the chocolate as properly tempered when the temperindex stays from 4.0 to 6.0. The formulations temperindex ranged from 4.6 to 6.0. After the temper, the chocolate was kept in a double boiler at 30 °C to melt any unstable crystals. The product remained at the ideal moulding temperature until being moulded either into acetate moulds  $(2.3 \times 2.8 \times 1.5 \text{ cm})$  to form round 10 g pieces for sensory evaluation, or into bars in rectangular polyethylene moulds  $(8.5 \times 2.5 \times 0.7 \text{ cm})$  for rheological tests. The moulds were agitated on a vibrating table (JAF Inox, Tambaú, Brazil) to remove air bubbles and placed in a cooling tunnel (Siaht, Jundiaí, Brazil) programmed for temperature variation every 11 min up to 11 °C until the product solidified.

The chocolate pieces were unmoulded, wrapped with aluminium foil and kept at 20 °C for 5 days for

instrumental analysis and for 60 days for sensory evaluation.

#### Texture determination

The *snap test* was performed with a texturometer TA.XT2i with a three point bend ring – HDP/3PB probe (Stable Micro Systems, Surrey, UK). This probe comprised two metallic, parallel bars and a third bar coupled to the machine arm, which descended vertically in predetermined speeds, causing sample fracture. The programmed test conditions were 3.0 mm s<sup>-1</sup> as the pretest speed, 1.7 mm s<sup>-1</sup> as the test speed and 10 mm s<sup>-1</sup> as the post-test speed.

Ten samples of each formulation were fractured, and the mean values of hardness, expressed as the breaking strength (g), were registered.

#### Sensory acceptance

The tests were conducted in individual cabinets illuminated with white light. The chocolate pieces were served at room temperature (20–24 °C), unwrapped on plastic plates numbered with three random digits. Mineral water at room temperature and diced peeled apple were served between samples.

The evaluation group was composed of fifty nine panellists, eighteen men and forty-one women, which is higher than the number of 52 recommended by Hough *et al.* (2006) for these assessment conditions. The panellists were between 18 and 40 years old and had undergraduate or graduate education (92%), and 79% of them consumed chocolate daily or weekly.

Eight samples were presented in two sessions to avoid panellist sensory fatigue. An 11-point hybrid hedonic scale with verbal terms in the extremes, beginning with 0 – strongly disliked and finishing with 10 – strongly liked, was used as the evaluation instrument for each sample (Villanueva *et al.*, 2005).

#### Scanning electron microscopy

Scanning electron microscopy (SEM) (Quanta-200; FEI Ltd., Eindhoven, The Netherlands.) was used to characterise the structures of the chocolates.

The samples were sectioned and fixed by immersion in a 3% solution of 0.1 M glutaraldehyde for 24 h at 4 °C and then washed two times by immersion in 0.1 M phosphate buffer for 5 min at 4 °C.

Subsequently, they were transferred to 1% osmium tetroxide and immersed for 7 days at 4 °C, then dehydrated in ethanol successively with 10, 80, 90 and 100 GL for 10 min at 4 °C. The samples were then dried in a dehydrator and coated with a gold peel 30 nm thick in a Sputter Coater (SCD 050; Leica Microsystems, Vienna, Austria).

Formulations CB (no fibre addition), IN10 (10 g/ 100 g of inulin) and BG10 (10 g/100 g of  $\beta$ -glucan concentrate) were chosen for the micrographs because they represented the most extreme design conditions and, consequently, allowed better visualisation and comparison of the formed microstructures.

#### Statistical analysis

Mathematical models based on Scheffé's canonical model were fitted to the texture and sensory acceptance results. Analysis of variance (ANOVA) at a 5% significance level (*F*-test) was performed to find the adjusted determination coefficient (adjusted  $R^2$ ). To study the significance of individual effects on the variable response, the independent variables were adjusted to a 5% significance level ( $P \le 0.05$ ). Data analysis and graph plotting were performed using STATISTICA version 10.0 software (Statsoft, 2010).

#### **Results and discussion**

#### Texture

Hardness describes the stiffness of chocolate and is directly related to sensory perception during consumption, which is dependent on the phase transition that occurs in the mouth. Higher breaking strength values are desirable in chocolates because they provide shine, durability against thermal and physical damage and appropriate mouth feel and melting behaviour (Vissotto *et al.*, 2005; Afoakwa *et al.*, 2009a; Shah *et al.*, 2010).

The obtained breaking strength values ranged from 5059 g (Formulation BG3.2 + IN3.2) to 6125 g (Formulation CB), as shown in Table 3. The chocolates with added fibre (inulin and/or  $\beta$ -glucan concentrate) had lower hardness values than did the control formulation.

Formulation CB, made with maltitol and no fibre addition, presented a hardness value close to the formulation C with saccharose. The texture of sugar-free chocolates with maltitol was evaluated by Vissotto *et al.* (2005), who also observed a breaking strength similar to that of chocolates produced with saccharose.

The adjusted model for the breaking strength results is shown in Table S1. The adjusted  $R^2$  of 80% and no significant lack of fit indicated good predictive power. Analysis of variance of the polynomial regression demonstrated that the three independent variables significantly contributed to the hardness.

It was observed that increasing cocoa butter concentrations resulted in increased hardness (Fig. 1), an effect also observed by Do *et al.* (2007). With the increase in the lipid phase, the solid particles became more scattered, causing a lower flow resistance and

Formulation*	Inulin†	β-glucan concentrate <sup>†</sup>	Cocoa butter <sup>†</sup>	Breaking strength (g) <sup>‡</sup>	Global acceptance <sup>‡§</sup>	Texture acceptance <sup>‡§</sup>	Flavour acceptance <sup>‡§</sup>
СВ	0.0	0.0	20.0	6125 ± 373	7.97 ± 1.11	8.25 ± 1.39	8.02 ± 1.05
IN10	10.0	0.0	10.0	5788 ± 390	7.74 ± 1.44	7.46 ± 1.75	7.89 ± 1.38
BG10	0.0	10.0	10.0	$5395 \pm 577$	$\textbf{6.44} \pm \textbf{2.03}$	$\textbf{6.28} \pm \textbf{1.87}$	$\textbf{6.48} \pm \textbf{2.09}$
BG5	0.0	5.0	15.0	$5715\pm546$	$7.54\pm1.55$	$\textbf{7.48} \pm \textbf{1.51}$	$7.64\pm1.58$
IN5	5.0	0.0	15.0	$5489\pm499$	$\textbf{8.16} \pm \textbf{1.43}$	$8.11\pm1.55$	$\textbf{8.17}~\pm~\textbf{1.32}$
BG5 + IN5	5.0	5.0	10.0	$5110\pm564$	$7.14\pm1.67$	$\textbf{6.91} \pm \textbf{1.93}$	$\textbf{7.26} \pm \textbf{1.71}$
BG3.2 + IN3.2	3.2	3.2	13.6	$5059\pm266$	$8.11\pm1.11$	$7.55\pm1.50$	$8.06\pm1.12$
BG3.2 + IN3.2'	3.2	3.2	13.6	$5180\pm204$	-	-	_
Control	0.0	0.0	20.0	$5971 \pm 509$	$\textbf{8.29}\pm\textbf{1.03}$	$\textbf{8.11} \pm \textbf{1.33}$	$8.19\pm1.05$

Table 3 Texture and acceptance attributes of chocolate formulations

\*CB, cocoa butter; IN, inulin; BG, β-glucan. The numbers in the samples codes represent the concentration of the ingredient in the chocolate formulation.

<sup>†</sup>Contents in chocolate formulation (g/100 g).

<sup>‡</sup>Mean  $\pm$  standard deviation of replicates.

 $^{\$}0$  = strongly disliked until 10 = strongly liked.

less interference in the crystallisation process, which resulted in harder chocolates (Ziegler & Hogg, 2009; Beckett, 2009).

The opposite effect was caused by  $\beta$ -glucan concentrate and inulin, which decreased the hardness as their concentrations were increased up to 5 g/100 g of the chocolate formulation (0.5 as pseudo component), and then increased the hardness at higher concentrations



**Figure 1** Adjusted surfaces for the breaking strengths of the mixtures containing inulin,  $\beta$ -glucan concentrate and cocoa butter, expressed as pseudocomponents. The experimental area is delineated by the sample points.

(0.75 and 1.0 as pseudo components). However, all formulations with fibre addition were less hard than the chocolates without fibre addition (Control and CB).

Rheological properties have a direct influence in texture. Harder chocolates are obtained when the melted product presents lower plastic viscosity, and when the viscosity increases, the final product will became softer. These properties of the studied samples were previously tested (Rezende *et al.*, 2014). The lower Casson plastic viscosity (2.19 Pa s) was found in CB that corresponds to the actual higher breaking strength and the higher (11.59 Pa s) in BG10, which have a low breaking strength value. Casson shear stress ranged from 0.26 Pa in CB to 22.92 Pa in BG10 following the same behaviour.

In Fig. 1, it can be observed that lower hardness values were observed for the formulations with less cocoa butter and intermediate concentrations of inulin and  $\beta$ -glucan. This effect can be explained by the augmented amount of particles in the solid phase caused by fibre addition. The lipid phase coats the particle surfaces and decreases their interactions; thus, as the coating decreases, the hardness also decreases (Beckett, 2009).

Lee *et al.* (2009) also observed that the partial substitution of cocoa butter with  $\beta$ -glucan concentrate causes a decrease in chocolate hardness and suggested that this substitution may influence the tempering process, which is directly related to the texture of the final product, causing softening.

Different from this study, which substitute sucrose by maltitol and add inulin as fibre enrichment in the maximum concentration of 10 g/100 g, Aidoo *et al.* (2014) tested the hardness when sucrose was



**Figure 2** Adjusted surfaces for the studied acceptance attributes. (a) Texture, (b) flavour and (c) global acceptance of the mixtures containing inulin,  $\beta$ -glucan concentrate and cocoa butter, expressed as pseudocomponents. The experimental area is delineated by the sample points.

substitute by inulin, achieving 48 g/100 g. In this higher concentrations, they verify that inulin increases hardness.

#### Sensory acceptance

Analysis of variance of the polynomial regression demonstrated that the three independent variables significantly contributed to the flavour, texture and global acceptance (Table S1).

The results obtained for the three attributes were very similar (Table 3), indicating that the panellists detected the formulation changes and were able to relate them to their sensory perceptions of the stated attributes.

An evaluation of the influence of the lipid phase promoted by functional ingredient addition on sensory acceptance has not been reported in the literature, but we hypothesised that the sensory perceptions would be related to the changes in texture caused by the substitution of ingredients.

Increasing the cocoa butter content enhanced the acceptance score (Fig. 2). This relationship is widely accepted in chocolate production, as cocoa butter provides rheological properties that promote proper hardness, mouth feel and swallow (Beckett, 2009).

The inverse relationship was observed for the inulin and  $\beta$ -glucan levels; increasing levels of these additives diminished the sensory attributes of the chocolates. The inclusion of these ingredients increased the amount of solid particles, decreasing the hardness, as observed in Fig. 1. Consequently, the melting of chocolate in the mouth was slowed.

According to Beckett (2009), the intensity of the perceived flavour would also decrease as a result because it is dynamically altered by the time that the chocolate takes to melt when it is manipulated and mixed with saliva during mastication.

The chocolate that had half of the cocoa butter quantity substituted for  $\beta$ -glucan concentrate (Formulation BG10) contained more solid particles than did the chocolate that had half of the cocoa butter quantity substituted for inulin (Formulation IN10). This influenced the sensory analysis of these samples, with lower scores for all analysed attributes (close to 6.0) observed for the formulation with a higher level of  $\beta$ -glucan.

Inulin is a bulk agent that improves the taste and melting of chocolate in the mouth (Shah *et al.*, 2010). This synergy with the product explains why inulin had a lower effect on the sensory acceptance.

Even at the highest level of inulin addition, the acceptance scores had small drops: 8.4–7.5 in texture, 8.0–7.9 in flavour and 8.0–7.8 in global evaluation.

Golob *et al.* (2004) also verified that the sucrose substitution of sucrose with inulin in milk chocolate formulations did not result in decreased sensory acceptance.

#### Scanning electron microscopy

Figure S1a presents the microstructure of Formulation CB, with no fibre addition. A well-defined crystalline network resulting from the coating of the solids by cocoa butter and efficient tempering procedures can be observed. This structure is similar to that observed by Afoakwa *et al.* (2009b) in micrographs of properly tempered chocolate, where the crystalline network was densely distributed. The presence of fewer connections between the crystals can be explained by the fact that the authors used saccharose in their formulation, while in this work maltitol was utilised, which is a polyol that is less crystalline than saccharose (Konar, 2013).

The crystalline network was significantly altered in the micrograph of formulation IN10, in which half of the cocoa butter was substituted with inulin (Fig. S1b). The presence of spherical structures not covered by cocoa butter can be observed, similar to the inulin images captured by Rosell *et al.* (2009), demonstrating that the amount of cocoa butter added was insufficient to coat all the solid particles in the formulation.

However, due to the reduced molecular size of inulin and to the good processing of the formulation, the sensory characteristics and hardness were not compromised, as discussed above.

In Formulation BG10, the substitution of half of the cocoa butter with  $\beta$ -glucan concentrate seriously compromised the crystalline network, which resulted in decreased hardness and acceptance of the product (Fig. S1c).

The presence of a large amount of particles deposited on the crystallised portions revealed that the coating of the solids was more affected than it was in Formulation IN10 containing inulin. Due to the difference in density of these two ingredients, the volume of  $\beta$ -glucan concentrate added was larger than that of inulin, and the quantity of particles incorporated into the formulation was also larger.

Among these particles, uncoated fibrous structures characteristic of  $\beta$ -glucan can be observed, as also reported by Rosell *et al.* (2009), in addition to spherical structures that can be attributed to starches from the concentrate or proteins from the other ingredients.

## Conclusions

The development of sucrose-free chocolates enriched with fibre was shown to be possible, and the substitution of up to 50% of the cocoa butter content with inulin and/or with  $\beta$ -glucan concentrate resulted in products with good sensory acceptance.

Both fibres reported in this study decreased the chocolate hardness, and the addition of  $\beta$ -glucan in the highest tested concentration (10 g of  $\beta$ -glucan concentrate in 100 g of chocolate) significantly decreased the sensory quality of the resulting chocolate.

The partial substitution of cocoa butter with fibre changed the chocolate microstructure due to the lack of full coverage of the solid particles in the formulations.

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### References

- Afoakwa, E.O., Paterson, A., Fowler, M. & Vieira, J. (2008). Relationship between rheological, textural and melting properties of dark chocolate as influenced by particle size distribution and composition. *European Food Research and Technology*, **227**, 1215–1223.
- Afoakwa, E.O., Paterson, A., Fowler, M. & Vieira, J. (2009a). Microstructure and mechanical properties related to particle size distribution and composition in dark chocolate. *Journal of Food Science and Technology*, **44**, 111–119.
- Afoakwa, E.O., Paterson, A., Fowler, M. & Vieira, J. (2009b). Influence of tempering and fat crystallisation behaviours on microstructural and melting properties in dark chocolate systems. *Food Research International*, **42**, 200–209.
- Aguilera, J.M. (2005). Why food microstructure? *Journal of Food Engineering*, **67**, 3–11.
- Aidoo, R.P., Afoakwa, E.O. & Dewettinck, K. (2014). Optimization of inulin and polydextrose mixtures as sucrose replacers during sugar-free chocolates manufacture – Rheological, microstructure and physical quality characteristics. *Journal of Food Engineering*, 126, 35–42.
- Beckett, S.T. (2009). Chocolate flow properties. In: *Industrial Chocolate Manufacture and Use*, 4th edn. (edited by S.T. Beckett). Pp. 224–246. York, UK: Wiley-Blackwell.
- BeMiller, J.N. & Huber, K.C. (2007). Carbohydrates. In: *Fennema's Food Chemistry*, 4th edn. (edited by S. Damondarian, K.T. Parkin & R. Owen). Pp. 83–154. Boca Raton, FL: CRC Press.
- Costell, E. & Durán, L. (2005). Food texture: sensory evaluation. In: *Food Engineering: Encyclopedia of Life Support Systems* (English version by G.V. Barbosa-Cánovas). Pp. 238–256. Oxford, UK: EOLSS.
- Do, T.L., Hargreaves, J.M., Wolf, B., Hort, J. & Mitchell, J.R. (2007). Impact of particle size distribution on rheological and textural properties of chocolate models with reduced fat content. *Journal of Food Science*, **72**, E541–E552.
- Farzanmehr, H. & Abbasi, S. (2009). Effects of inulin and bulking agents on some physicochemical, textural and sensory properties of milk chocolate. *Journal of Texture Studies*, **40**, 536–553.
- Frank, A. (2002). Technological functionality of inulin and oligofructose. *British Journal of Nutrition*, **87**, S287–S291.
- Golob, T., Micovic, E., Bertoncelj, J. & Jamnik, M. (2004). Sensory acceptability of chocolate with inulina. *Acta Agriculturae Eslovenica*, **83**, 221–231.
- Gomes, C.R., Vissotto, F.Z., Fadini, A.L., Faria, E.V. & Luiz, A.M. (2007). Influência de diferentes agentes de corpo nas características reológicas e sensoriais de chocolates diet em sacarose e light em calorias. *Ciência e Tecnologia de Alimentos*, **27**, 613–623.
- Hough, G., Wakeling, I., Mucci, A., Chambers IV, E., Gallardo, I.M. & Alves, L.R. (2006). Number of consumers necessary for sensory acceptability tests. *Food Quality and Preference*, **17**, 522– 526.

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- Konar, N. (2013). Influence of conching temperature and some bulk sweeteners on physical and rheological properties of prebiotic milk chocolate containing inulin. *European Food Research and Technol*ogy, 236, 135–143.
- Lee, S., Biresaw, G., Kinney, M.P. & Inglett, G.E. (2009). Effect of cocoa butter replacement with β-glucan-rich hydrocolloid (c-trim 30) on the rheological and tribological properties of chocolates. *Journal of the Science of Food and Agriculture*, **89**, 163–167.
- Lim, J., Inglett, G.E. & Lee, S. (2010). Response to consumer demand for reduced-fat foods; multi-functional fat replacers. *Japan Journal of Food Engineering*, **11**, 163–168.
- Melo, L.L.M.M., Bolini, H.M.A. & Efraim, P. (2009). Sensory profile, acceptability, and their relationship for diabetic/reduced calorie chocolates. *Food Quality and Preference*, **20**, 138–143.
- Rezende, N.V., Benassi, M.T., Vissotto, F.Z., Augusto, P.P.C. & Grossmann, M.V.E. (2015). Mixture design applied for the partial replacement of fat with fibre in sucrose-free chocolates. *LWT-Food Science and Technology*, **62**, 598–604. (Issue 1, Part 2)
- Rosell, C.M., Santos, E. & Collar, C. (2009). Physico-chemical properties of commercial fibres from different sources: a comparative approach. *Food Research International*, **42**, 176–184.
- Rousseau, D. (2006). On the porous mesostructure of milk chocolate viewed with atomic force microscopy. *LWT-Food Science and Technology*, **39**, 852–860.
- Shah, A.S., Jones, G.P. & Vasiljevic, T. (2010). Sucrose-free chocolate sweetened with *Stevia rebaudiana* extract and containing different bulking agents – effects on physicochemical and sensory properties. *International Journal of Food Science and Technology*, **45**, 1426–1435.

- Statsoft. (2010). *Statistica Version 10.0 for Windows*. Tulsa: Statsoft Inc. Software.
- Villanueva, N.D.M., Petenate, A.J. & Silva, M.A.A.P. (2005). Performance of the hybrid hedonic scale as compared to the traditional hedonic, self-adjusting and ranking scales. *Food Quality* and Preference, 16, 691–703.
- Vissotto, F.Z., Gomes, C.R. & Batista, B.J.L. (2005). Caracterização do comportamento reológico e da textura de chocolates sem sacarose. *Brazilian Journal of Food Technology*, 8, 107–111.
- Ziegler, G.R. & Hogg, R. (2009). Particle size reduction. In: *Industrial Chocolate Manufacture and Use*, 4th edn. (edited by S.T. Beckett). Pp. 169–192. York, UK: Wiley-Blackwell.

#### **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Scanning electron microscopy (SEM) fracture micrograph of the formulations: (a) CB (without fibre), (b) IN10 (50% of the cocoa butter substituted with inulin) and (c) BG10 (50% of the cocoa butter substituted with  $\beta$ -glucan concentrate).

**Table S1.** Coefficients and analysis of variance of the models adjusted to the rheological parameters and sensory acceptance.