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The Impact of Sugar Particles Size and Natural Substitutes for the Replacement of Sucrose and Fat in Chocolate Brownies: Sensory and Physicochemical Analysis

Aislinn M. Richardson, Andrey A. Tyuftin, Maurice G. O' Sullivan, Kieran N. Kilcawley, Eimear Gallagher and Joseph P. Kerry

Abstract — **As fat contributes important textural properties such as lubricity and tenderness to cakes, it is plausible to focus on ways to increase the perception of these properties with the aim of creating the illusion of a higher fat. The utilisation of small sugar particles has been shown to increase the moist and soft texture of Chocolate Brownies. The present study assessed three different sugar particle sizes in their ability to create the illusion of fat content and therefore their ability to permit fat replacement (FR) in this product. The unground commercial sugar (200-5181 µm) was used as the control (UC) and two of its sieved sugar separates, Large ($L_{924\text{-}1877\ \mu m}$) and Small ($S_{459\text{-}972\ \mu m}$) were investigated. For each, fat was replaced using pureed black beans. The most accepted sample was used for sucrose replacement (SR) using inulin and *Rebaudioside* A. (*Reb* A.). Samples containing the smallest sugar fraction with 25% FR were most significantly associated overall acceptability (OA) (p<0.01). The application of small sugar particles did not significantly negatively affect OA or liking of samples at a level of 75% FR compared to the other two sugar fractions. The utilisation of small sugar particles (459-972 µm) in the preparation of baked goods could aid baking & industry professionals in reducing the fat content of cake-like products.**

Index Terms — **Chocolate Brownies, Sugar reduction, Fat reduction, Bakery products.**

I. INTRODUCTION

Cakes, biscuits, and confectionery products such as sweets, snacks, and popcorn account for 12% of the total fat intake of Irish adults aged between 18 and 64 [1]. Sweet products comparable to these also account for a high percentage of the intake of total and added sugar in European adults and are the largest contributor to the intake of both according to a recent review [3].

According to data obtained from the North/South food consumption survey, Irish adults are exceeding recommendations for both fat and sugar intake [1]. This survey determined that Irish adults consume a total of 61.9 g of added sugar per day. This is high considering the recommendations made by organisations such as the World Health Organisation (WHO) (<50 g/day for the average adult) and the American Heart Association (AHA) (<25 g/day for women and <37.5 g/ day for men) for added sugar intake [2], [3].

Chocolate Brownies are a well-liked desert worldwide. They can be described as a cake-like bar, classified by a high content of butter, sugar, dark chocolate and a low content of flour. Typically, no leavening agents are added to the batter to create a more fudge-like, dense texture. Foods that are rich in fat and sugar are highly palatable [4], [5]. The presence of fat and sugar in food products yields positive hedonic responses to attributes such as aroma, texture, and flavour [6]. The risk of overconsumption of foods containing a mixture of fat and sugar is high as positive hedonic responses may override metabolic responses such as satiety [7]. Cake-like products such as Chocolate Brownies are high in fat and sugar and are typically energy rich and nutrient poor food products [8]. Thus, the consumption of these products can lead to dietary imbalances which have been associated with diseases such as obesity [9].

In Ireland 37% of adults are overweight and a further 23% are obese according to the Healthy Ireland survey [10]. It has been estimated that roughly 90% of type 2 diabetes cases worldwide are due to excess weight [11]. Other serious implications of obesity include increased risk of cardiovascular problems, certain cancers, and gall bladder disease, among others [12]. According to the WHO, over half a million people died in 2002 from obesity related complications [9].

Therefore, the reduction of sugar and fat in cake-like products such as Chocolate Brownies, could be a significant development in reducing the dietary intake of both sugar and fat. Furthermore, the replacement of fat and sugar with functional ingredients such as fibres could increase the nutritional quality of these well-liked products. However, successful replacement of fat and sugar presents a great challenge for food researchers and industry because of the multiple functions performed by these ingredients. Fat plays a vital role in the tenderization of cakes and also adds

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lubricity to the texture by coating the protein and starch particles, thereby interfering with the protein matrix [13]. Fat also emulsifies liquid in cake production which adds moisture and softness to the product [14]. Sugar is responsible for the sweetness of cakes [15] and also binds moisture [16]. Sugar inhibits or reduces gluten development during cake batter mixing by competing with gluten proteins for water and thus, acts as a tenderiser of baked goods [17].

The objective of this study was to assess the impact of different sugar particle sizes and the utilisation of natural substitutes for the replacement of fat and sucrose in production of Chocolate Brownies and to investigate their sensory and physical properties.

II. Materials and Methods

Food ingredients used in this trial, included; Light golden soft brown sugar (1.1% moisture, 98% sucrose, cane molasses & invert sugars, Siucra brand, UK); Irish Creamery butter (81% total fats, 65.4% of which were saturated & 15.1% moisture, Dunnes stores, Ireland); Black beans (18% carbohydrate, 0.5 % of which were sugars, 8.2% protein, 6.4 % fibre, 0.8 % fat, 0.2% of which were saturated, 0.02% sodium, 0.05% salt & 73.2% moisture (after being drained and pureed), Suma brand, UK); Inulin (89% fibre & 8% sugar, Bioglan brand, Austrailia); *Reb* A. (Bulk Powders brand, Ireland); Cream plain flour (82.7% carbohydrate, 2% of which were sugars, 11.7% protein, 3.4% fibre, 1.4% fat, & 0.81% salt, Odlums, Ireland); Free range eggs (Upton brand, Ireland); Dark chocolate (55.8% carbohydrate, 97.2% of which sugars, 34.7% fats, 3.6% protein & 0.1% salt, Homecook wonder bar, Ireland). Food products were all purchased from a local supermarket unless stated otherwise and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation.

A. Sieving & Measurement of Particle Size

Sugar was separated into different size fractions by grinding and sieving (mechanically sieved through a sequence of sieves (90, 125, 150, 180, 212 and 355 µm) set) and then measured according to the method employed in Richardson et al. [19]. For the purpose of this experiment the following three sugar sizes were selected: the unground control sugar fraction (UC), a large sugar fraction (L), ($L_{924\text{-}1877\ \mu m}$) and a small sugar fraction (S), ($S_{459\text{-}972\ \mu m}$).

B. Chocolate Brownie Treatments

The formulation used for the preparation of the control chocolate brownie treatment was based on conversations had with local bakeries, cookbook recipes and associated websites. Three separate batches of brownies for all experimental treatments (16) were formulated and manufactured using recipes outlined in Table 1.

TABLE 1: Formulation of different Chocolate Brownies treatments

	%										
	Sugar particle size (µm)			Sucrose	Butter	Pureed BB	Eggs	Flour	Dark chocolate	Inulin	Stevia
Replace-ment levels (%)	200-5181	924-1877	459-972								
	UC0/0	L0/0	S0/0	27.9	19.6	0	20.1	12.8	19.6	0	0
	UC25/0	L25/0	S25/0	27.9	14.7	4.9	20.1	12.8	19.6	0	0
	UC50/0	L50/0	S50/0	27.9	9.8	9.8	20.1	12.8	19.6	0	0
	UC75/0	L75/0	S75/0	27.9	4.9	14.7	20.1	12.8	19.6	0	0
Sucrose replace-ment	-	-	S75/0	27.9	4.9	14.7	20.1	12.8	19.6	0	0
	-	-	S75/25	20.9	4.9	14.7	20.1	12.8	19.6	7.0	0.018
	-	-	S75/50	14.0	4.9	14.7	20.1	12.8	19.6	13.9	0.03
	-	-	S75/75	7.0	4.9	14.7	20.1	12.8	19.6	20.9	0.05

UC; Unground commercial fraction of sugar with a particle size range of 200-5181µm, L; Large fraction of sugar with a particle size range of 924-1877 µm, S; Smallest fraction of sugar with a particle size range of 459-972 µm. The first digit represents the fat replacement level, and the second digit represents the sucrose replacement level of samples.

During the first phase of this trial a control treatment with 0% fat replacer (FR) was prepared for each sugar size group, UC, L & S. For each sugar size, fat was replaced by increments of 25% using pureed black beans. Thus, 12 treatments were formulated, and samples were identified as follows: UC/0, L/0, & S/0, UC/25, L/25 & S/25, UC/50, L/50 & S/50 and UC/75, L/75 & S/75. Following this part of the study the most accepted sugar particle size and FR level combination were chosen and used in the next phase. Four more formulations were prepared where sucrose was replaced sequentially by increments of 25% in reduced fat Chocolate Brownie samples using a combination of inulin and *Reb* A. The samples were identified as follows; S75/0, S75/25, S75/50 and S75/75, where the first letter denotes the sugar particle size used, the first digit represents the FR level employed and the last digit represents the level of SR in each sample.

C. Reb A. Concentration Adjustment

A ranking test was used to determine the concentration of *Reb* A. needed to replace the sweetness concentration of sucrose, ensuring iso-sweetness. Ranking tests were carried out twice using 21 assessors. Concentration adjustments for *Reb* A. were carried out according to the method of Zahn et al. [18] using the same concentrations of stevia (0.06-0.16 g/l) and 24g/L sucrose. One-way ANOVA was used to compare the means of the data obtained for each solution. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA).

D. Chocolate Brownie Preparation

Brownies were prepared according to the method described in Richardson et al. [19] and adapted for fat and sucrose replacement. Dark chocolate and butter were melted in a heat stable bowl in a microwave oven. The melted mixture was stirred before sugar was added. Eggs were beaten in a separate bowl and added to the mixture. All of the ingredients were stirred until flour was sieved into the mixture. Mixture was stirred by hand until smooth. Preparation was adapted to accommodate for fat replacement initially and then sucrose replacement. Black beans were drained and pureed in a Stephan mixer (UMC-5 Stephan u. Sohner & Co, Hameln, Germany) at 21 RPM for 5 mins before being added to the batter in partial replacement of butter. For sucrose replacement inulin and *Reb* A. were mixed in a separate bowl before being added to the batter in partial replacement of sucrose. The batter was poured into tinfoil trays and batches were baked for 30 min. Batches of brownies were left to set for 30 min in the tray before being removed and cut into Individual brownie pieces. Chocolate Brownies were placed on a rack for cooling for one hour before being removed and placed into plastic containers for storage prior to testing.

E. Sensory analysis

Sensory Acceptance Testing

Sensory acceptance testing (SAT) [20], [21] was carried out in the panel booths of the sensory science laboratory, food science building, University College Cork according to international standards (ISO 11136:2014). Using 25 untrained assessors who were familiar with the products being tested (n=25), SAT took place over six separate sessions as three independent trials were carried out for both phases of this study. To accommodate for the analysis of a large number of treatments during fat replacement trials (12), in duplicate, each SAT session took place over three days so that all participants tasted every sample twice. According to [20], having participants return to evaluate all products produces better results than balanced incomplete block designs. For sucrose replacement trials, SAT sessions for each independent trial were carried out in one day as only 4 treatments were tested in duplicate. Samples (2×2×2 cm) were assigned a randomised three-digit code and sessions were carried out at room temperature under white light. Participants were instructed to use the water provided to cleanse their palates between tastings and used the following hedonic descriptors to rate their degree of liking; appearance, flavour, texture, colour, and aroma liking. Assessors were asked to indicate their degree of liking for samples on a 9-point, numbers only hedonic scale. Words were only used to anchor the scale at both ends with the term ‘extremely dislike’ on the far left end of the scale and the term ‘extremely like’ on the far right hand-side of the scale. Overall acceptability (OA) of samples was also determined using this scale. As three independent trials were carried out for each phase, using 25 untrained assessors, 150 responses were collected for each sample (25 + 25+ 25 x 2).

Optimized Descriptive Profiling (ODP)

Optimized Descriptive Profiling (ODP) [22] was carried out in the panel booths of the sensory science laboratory, food science building, University College Cork. A separate panel of 21 assessors (n = 21) all of whom had previous experience with descriptive analysis, were trained and participated in this separate descriptive analysis [22]. ODP sessions ran concurrently with SAT sessions and therefore took place over six weeks as three independent trials were carried out for both phases of this study using the same panel. Sensory descriptors were selected from panel discussion as the most appropriate and reflected the main variation in the samples profiled. The consensus list of intensity descriptors (Table 2) was measured on a 10 cm continuous line scale with the term “none” used as the anchor point for the 0 cm end of the scale and the term “extreme” being used as the anchor point for the 10 cm end of the scale. The samples (2x2x2cm) were served coded in randomised order and presented simultaneously to assessors [23].

TABLE 2: CONSENSUS LIST OF INTENSITY DESCRIPTORS AND DEFINITIONS OF DESCRIPTORS USED IN RANKING DESCRIPTIVE ANALYSIS (RDA) OF CHOCOLATE BROWNIES

Attributes	Definition
Touch	
Springiness	The impact of applying physical force to the original shape of the sample of chocolate brownie.
Appearance	
Crust darkness	Degree of darkness of crust
Texture	
Hardness	Force needed to compress sample in mouth
Moisture	Wet texture in mouth
Dense	Heavy, rich, wet-like texture
Flavour	
Sweet taste	Taste sensation associated with sucrose
Butter flavour	Flavour sensation associated with butter; creamy mouth-feel and buttery aroma
Chocolate flavour	Intensity of cocoa flavour
Off flavour	Flavour not associated with Chocolate brownies

F. Physicochemical Analysis

Physicochemical analysis was carried out during sucrose replacement trials on the following samples: S75/0, S75/25, S75/50 and S75/75. For the purpose of examining the physical and compositional impact of a 75% fat replacement on chocolate brownie samples, physicochemical properties were also obtained for the S0/0 sample.

Texture profile analysis (TPA)

As outlined previously, three independent trials were carried out for all treatments. Two Chocolate Brownies (45×45×30 mm) from the centre of each batch tray were used for texture analysis. Thus, results obtained for TPA represent a mean of 6 values (3×2 = 6). Texture profile analysis (TPA) was carried out on samples using a Texture Analyser 16 TA-XT2I (Stable Micro Systems, Surrey, UK). A 50% double compression test was carried out on each sample with a 75mm diameter flat-ended cylindrical probe (P/75), at a speed of 1mm/s with a 5 sec waiting time between the two cycles. This was carried out in accordance with the method of Martínez-Cervera et al. [17].

Colour

Two Chocolate Brownies (45×45 mm) from the top right of each batch tray were used for colour analysis. Crust and crumb colour characteristics were assessed by the CIE $L^*a^* b^*$ method. Lightness L^* was defined by means of a Minolta CR-200B Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). The L^* parameter ($L^*=0$ [Black], $L^*= 100$ [White]) for crust was measured at two separate points directly from the top of each individual brownie sample. The brownie samples were cut horizontally to remove the crust and crumb colour was measured directly at two separate points. As two measurements for crust and crumb colour were taken for each individual sample and two samples were tested for each individual trial, of which there were three, crust and crumb colour values represent a mean of twelve measurements (2×2×3).

G. Statistical Analysis

Raw data obtained from sensory (hedonic & intensity) and physicochemical analysis was coded into Microsoft excel.

The significance of sensory and physicochemical properties in discriminating between the samples was analysed using ANOVA and Tukey's post-hoc test (SPSS statistics 20 software (IBM, Armonk, NY, USA). For fat optimisation, the relationship between the set of samples (12) and the set of sensory variables was determined by partial least squares (PLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). In the PLS regression only sensory properties that discriminated significantly between samples were used. The X-matrix was defined as the different sample treatments. The Y – matrix contained the significant sensory variables of the design. For sucrose optimisation the relationship between the set of sample treatments (X) and the set of sensory & physicochemical variables (Y) was examined by PLS regression. Again, only sensory, and physicochemical properties that discriminated significantly between samples (4) were used. Both the sensory and physicochemical data were normalised during pre-processing of the data by taking the logarithm to achieve uniform precision over the whole range of variation. Data was also standardised by dividing each variable (sensory & physicochemical) by its standard deviation. This process was necessary as the units of the studied variables were different. To achieve significant results for the relationships determined in quantitative PLS, regression analysis, coefficients were analysed by jack-knifing which was based on custom cross-validation and stability plots [24]. Statistical significance for the relationships analysed by PLS were defined as P<0.05-0.01 (significant), P<0.01-0.001 (highly significant) and P<0.001 (extremely significant).

TPA and proximate composition data were presented as a mean of six values ± standard deviation. Estimated fibre and sucrose content were presented as a mean of three values ± standard deviation. Colour (crust and crumb) data was presented as a mean of twelve values ± standard deviation. One-way ANOVA was used to compare the means of the data obtained from physicochemical analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA).

III. Results and Discussion

A. *Particle Diameter Distribution*

The particle diameter distribution of the three sugar fractions investigated in this study can be seen in Fig. 1.

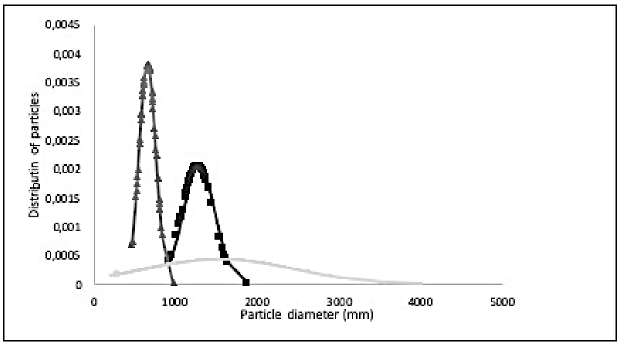

Fig. 1. Particle diameter distribution of unground control sugar fraction UC; ( ) and sugar-sieve separates; L ( ) and S ( ).

The UC sugar fraction had the widest particle diameter distribution as expected, with particles ranging from 200-5181 µm. The L sugar fraction had a particle diameter distribution of 924-1877 µm and the smallest sugar fraction (S) had the narrowest particle diameter distribution of 459-977 µm.

B. *Reb A. for Sucrose Replacement*

The sweetness rankings of six different concentrations of *Reb* A. and one standard solution of sucrose are presented in Table 3.

TABLE 3: Iso-sweetness of Rebaudioside A in Aqueous Solutions

Sweetener	Concentration (g/L)	Mean scores	Dilution factor
Reb A.	0.060	1.2^{a}	400
	0.069	5.4^{b}	350
	0.080	6.2^{c}	300
	0.096	6.3^{c}	250
	0.120	7.4^{d}	200
	0.160	9.4^{d}	150
Sucrose	24.0	5.6^{b}	n/a

[abcd] mean values (± standard deviation) in the same column bearing different superscripts are significantly different, P < 0.05. Concentrations and dilution factors obtained from [18].

The *Reb* A. solution containing 0.069 g/L did not obtain significantly different scores from the standard sucrose solution with regards to sweetness intensity. For this reason, a sucrose-to-*Reb* A. ratio of 1:350 was chosen. This means, for samples containing 25% SR which equates to a reduction of 62.5 g of sucrose in the formulation, 0.17 g of *Reb* A. was used to replace the sweetness (62.5/350). The same method was applied to samples containing 50 & 75% SR. Inulin was added on a weight by weight basis.

C. *Sensory Analysis*

1. Relationship between sensory variables and Brownie samples prepared with different sugar sizes and with increasing levels of FR.

The relationship between sensory properties (hedonic & intensity) (Y) and Chocolate Brownie samples prepared with three different sugar size fractions and with increasing levels of fat replacement (X) is visually represented by a Partial least squares regression plot (PLSR) shown in Fig. 2.

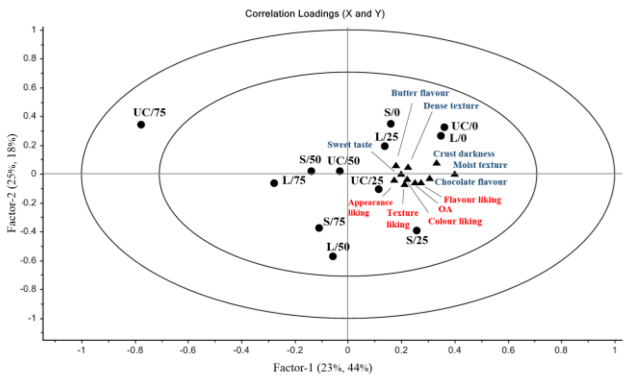

Fig. 2. Partial least squares regression (PLSR) plot for the relationship between Chocolate brownie samples prepared with three different sugar sizes (UC, L & S) and with increasing levels of fat replacement; 0, 25, 50 &75% ( ) and sensory terms ( ). Hedonic ( ) and Intensity sensory terms ( ).

The following intensity terms were left out of PLSR analysis because they did not significantly discriminate between samples: hard texture, springiness and off flavour. The hedonic term ‘aroma Liking’ was also omitted from PLSR analysis for the same reason. Most of the variation is shown in Factor-1 where 23% of the X data explains 44% of the data in Y. All intensity and hedonic sensory attributes are positioned in the inner circle of the upper and lower right quadrants. It is evident from the plot that a high correlation between all significant intensity attributes existed as seen by their close proximity to each other. These intensity attributes (Butter flavour, dense texture, crust darkness, moist texture, chocolate flavour and sweetness) were highly correlated with all significant hedonic parameters investigated in this study (Appearance, texture, colour, flavour liking and OA) also evident by their close proximity to each other. All samples containing 0% fat replacement in each sugar size (S/0, UC/0 and L/0) are positioned in close proximity with each other in the inner circle of the upper right quadrant. These samples were highly correlated with positive sensory attributes and hedonic parameters. Samples containing 25% FR in each sugar size (L/25, UC/25 and S/25) which are all situated on the right-hand side of the plot were also associated with positive intensity attributes and hedonic parameters. The sample containing the unground control sugar fraction with 75% FR (UC/75) made a significant contribution to Factor-2 on the plot and is positioned in the outer circle of the upper left quadrant. This sample was highly anti-correlated with all significant intensity attributes associated with hedonic parameters and OA. Samples containing the large sugar fraction with the same level of FR (75%) (L/75) were also anti-correlated with liking parameters and positive intensity attributes but not to the same extent as the UC/75 sample. The same can be said for the sample of Chocolate Brownies prepared with the smallest sugar fraction containing this level of FR (S/75) which is positioned in the inner circle of the lower left quadrant. Samples containing each sugar size with 50% FR were also slightly anti-correlated with intensity attributes associated with liking and OA.

To aid further understanding of the relationship between sensory terms and Chocolate Brownie samples, significance of estimated regression coefficients for the relationship between these two sets of variables can be seen in Table 4.

TABLE 4: SIGNIFICANCE OF ESTIMATED REGRESSION COEFFICIENTS (ANOVA VALUES) FOR THE RELATIONSHIP OF SENSORY TERMS (Y) AND CHOCOLATE BROWNIES PREPARED WITH DIFFERENT SUGAR SIZES (UC, L & S) AND WITH INCREASING LEVELS OF FR USING PBB

	Hedonics					Attribute intensity					
						Appearance	Texture		Flavour		
Sample	Appearance	Flavour	Texture	Colour	Overall acceptability	Crust darkness	Moist	Dense	Sweet taste	Butter flavour	Chocolate flavour
UC0/0	0.088	0.001***	0.044*	0.007**	0.002**	0.000***	0.000***	0.000***	0.018*	0.001***	0.000***
UC25/0	0.889	0.829	0.987	0.882	0.777	0.978	0.506	0.491	0.163	0.399	0.751
UC50/0	0.500	0.192	0.380	0.327	0.411	0.193	0.273	0.762	0.644	0.944	0.449
UC75/0	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***
L0/0	0.018*	0.091	0.051*	0.022*	0.003**	0.001***	0.000***	0.000***	0.000***	0.001***	0.001***
L25/0	0.688	0.257	0.555	0.484	0.321	0.032*	0.025**	0.082	0.487	0.107	0.242
L50/0	0.524	0.788	0.795	0.364	0.978	-0.001***	0.137	-0.039*	0.940	-0.047*	0.652
L75/0	-0.022**	-0.001***	-0.012**	-0.005**	-0.002**	-0.000***	-0.000***	-0.000***	-0.016*	-0.000***	-0.000***
S0/0	0.374	0.402	0.699	0.232	0.502	0.006***	0.000***	0.000***	0.000***	0.000***	0.000***
S25/0	0.003**	0.231	0.001***	0.000***	0.001***	0.013*	0.006**	0.107	0.684	0.623	0.001***
S50/0	0.120	0.998	0.188	0.145	0.168	0.569	0.329	0.095	0.219	0.292	0.088
S75/0	0.764	0.400	0.984	0.438	0.922	-0.001***	0.098	0.185	0.527	0.184	0.735

Significance of regression coefficients*=P≤0.05, **= P≤0.01, ***= P≤0.001 (–) indicates whether the relationship is negatively correlated.

Resembling results which are visually represented in the PLSR plot, the UC/75 sample was extremely significantly negatively associated with all liking parameters, and all positive attributes associated with liking and OA ($p<0.001$). The L75 sample was also extremely significantly negatively associated with flavour liking, crust darkness, moist & dense texture and butter & chocolate flavour ($p<0.001$), very significantly negatively associated with appearance, texture, colour liking & OA ($p<0.01$) and significantly negatively associated with sweet taste ($p<0.05$). After custom cross validation during PLSR analysis, the S/75 sample was only found to have an extreme significant negative correlation with crust darkness ($p<0.001$). This means all other important sensory properties and liking parameters were not significantly negatively affected up to a level of 75% FR in samples containing the smallest sugar fraction which was a very important result for this study.

As mentioned, intensity attributes investigated in this study were all highly correlated with each other. This was not surprising as the difficulty to detect fat in foods has been reported by Drewnowski et al. [25] who stated that no single attribute is correlated to fat content. Hence most intensity parameters investigated were significantly affected by a 75% replacement of fat with purred black beans. However, this was only observed for samples containing the UC & L sugar fractions. A reduction in perceived butter flavour with increasing levels of butter replacement in biscuits was reported by Laguna et al. [26]. Fats carry lipid-soluble flavour compounds [27] which could help to explain why perceived chocolate flavour was also affected at this level of FR. The combined effect of fat and sugar on sensory acceptability has been demonstrated previously by [4] and more recently by Biguzzi et al. [28] who found that perceived sweetness intensity declined with fat reduction in biscuits. Therefore, it is not surprising that sweetness intensity was affected at this level of FR in this study. Regarding the decrease in perceived moist texture, Lillford [29] confirmed that higher levels of fat increase the perceived intensity of moistness as the presence of fat reduces the need for saliva absorption, hence the decline of perceived moist texture here at a level of 75% FR in the UC and L sugar fractions.

All Samples containing 0% fat replacement (UC/0, L/0 & S/0) were extremely significantly positively correlated with crust darkness, moist & dense texture, and butter flavour &

chocolate flavour ($p<0.001$). The UC/0 sample was significantly associated with sweet taste intensity ($p<0.05$) but not to the same extent as the L/0 and S/0 samples which were extremely correlated with this attribute ($p<0.001$). Results obtained for perceived sweetness intensity were therefore in agreement with results obtained in Richardson et al. [19] where it was determined that the utilisation of different sugar sizes affected the perceived sweetness intensity of Chocolate Brownies. Although the UC/0 sample was the sample least associated with sweet taste out of all the samples containing 0% FR, it was the sample most correlated with flavour liking ($p<0.001$). Results obtained for the L/0 and S/0 samples in relation to flavour liking were not significant. It is plausible that the high correlation between these samples and sweet taste intensity negatively affected flavour liking of these samples. Previous research has shown that preference scores rise and then decrease with increasing levels of sucrose [4]. Although sucrose content remained constant in all samples during the first part of this study, the perception of sweet taste was higher in samples containing the L and S sugar fractions. In relation to liking parameters and OA, although the S/0 sample was significantly correlated with positive intensity attributes there was no significant relationship determined between this sample and liking parameters and OA compared to the UC/0 and L/0 samples.

With all of this said, the sample most significantly associated with OA was the sample containing the smallest sugar fraction with 25% FR ($p<0.001$). This sample was significantly associated with crust darkness ($p<0.05$) very significantly associated with moist texture ($p<0.01$) and extremely correlated with chocolate flavour ($p<0.001$). As a result, this sample was very significantly associated with appearance liking ($p<0.01$) and extremely associated with texture and colour liking ($p<0.001$). Although this sample was found to have no significant correlation with sweet taste or butter flavour, it appears that this sample contained the right balance of flavours and textural properties to drive liking and acceptability.

Therefore, the utilisation of smaller sugar particles improved OA of samples while permitting fat replacement up to a level of 25%. The application of small sugar particles did not significantly negatively affect OA or liking of samples at a level of 75% FR compared to the other two sugar fractions. As all intensity sensory properties correlated highly with each other it is hard to determine exactly how the utilisation of smaller sugar particles permitted this level of fat replacement compared to the UC and L sugar fractions. As the utilisation of this sugar fraction has been shown to increase the perception of moist texture, perhaps this attribute was maintained enough in samples containing 75% FR to maintain other sensory properties associated with liking and OA. Pureed black beans have previously been shown to successfully replace shortening in brownies up to a level of 90% FR as determined by [30]. For the above reasons it was determined that sensory properties associated with liking in this study were maintained enough by the smallest sugar fraction to allow for the successful replacement of fat by up to 75% and therefore this combination of sugar particle size and fat replacement level were chosen for proceeding with tests on sucrose reduction.

2. Relationship between sensory & physicochemical variables and reduced fat Chocolate Brownies prepared with increasing levels of SR

The second part of this study involved the sequential replacement of sucrose using a combination of inulin and *Reb* A. in reduced fat Chocolate Brownie samples. The relationship between sensory terms & physicochemical parameters (Y) and reduced fat Chocolate Brownie samples prepared with 0, 25, 50 and 75% SR (X) is visually represented by a PLSR plot in Fig. 3.

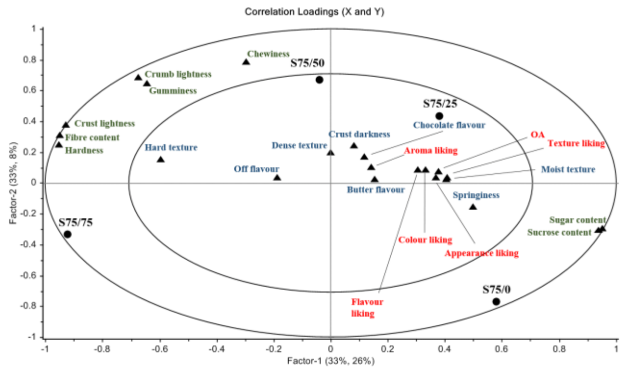

Fig. 3. Partial least squares regression (PLSR) plot for the relationship between fat reduced Chocolate brownie samples (75%) prepared with the smallest sugar size and with increasing levels of sucrose replacement; 0, 25, 50 and 75% (●) and sensory & physicochemical variables (▲). Hedonic (▬) and intensity (▬) sensory terms and physicochemical parameters (▬).

The sensory term 'sweet taste' was left out of PLSR analysis because it did not discriminate between samples which demonstrates the effectiveness of inulin and *Reb* A. as a substitute for sucrose in relation to this attribute. The following physical parameters and compositional properties were also excluded from PLSR analysis for the same reason; springiness (mm), cohesiveness, crust, and crumb redness (a*) and yellowness (b*) and moisture, fat, protein, ash, and carbohydrate content (%). Most of the variation is shown in Fator-1, where 33% of the X data explains 56% of the data in Y.

It is evident from the plot that the SC75/0 sample which is positioned in the outer circle of the lower right quadrant makes a very significant contribution to Factor-2. This sample was highly correlated with actual 'sugar content' as expected and as evident by their close proximity to one another on the plot. All significant hedonic sensory parameters which are positioned in close proximity with one another in the inner circle of the upper right quadrant were all correlated with one another (aroma, flavour, appearance, texture, colour liking and OA). The following intensity sensory properties are shown in close proximity with liking parameters and OA and are therefore drivers for the acceptability of samples: crust darkness, chocolate flavour, butter flavour and moist texture. Both the S75/0 and the S75/25 sample (situated in the inner circle of the upper right quadrant) were positively associated with these intensity attributes associated with liking parameters and OA. Although chocolate and butter content did not discriminate between samples in this part of the study these attributes were clearly more associated with samples containing either 0% or 25% SR. In a study conducted on chocolate flavoured milk it was found that reducing sugar content reduced the citation of the terms ‘sweet taste’ and ‘chocolate’ to describe the samples being tested [31]. Although ‘sweet taste’ did not discriminate between samples in our study the presence of sucrose increased the perception of chocolate flavour & butter flavour because of its flavour enhancing abilities, it is also important to consider the synergistic relationship between butter and sugar in cake products. The S75/75 sample which is situated in the outer circle of the lower left quadrant makes a significant contribution to Factor-1. This sample was highly anti-correlated with positive sensory properties and liking parameters located on the right hand side of the plot. This sample was positively associated with the following compositional parameters; fibre content (%), instrumental parameters; hardness (N), crust and crumb lightness (L*) gumminess (N) and sensory properties; hard texture & off-flavour. Sensory and instrumental results obtained for texture hardness therefore, correlated with each other as seen by their close proximity to one another on the PLS plot. The S75/50 sample which is situated in the inner circle of the upper left quadrant was also anti-correlated with positive sensory properties associated with liking and OA but not to the same extent as the S75/75 sample.

To aid further understanding of the relationship between these two sets of variables, significance of estimated regression coefficients is displayed in Table 5.

TABLE 5 (A-C): SIGNIFICANCE OF ESTIMATED REGRESSION COEFFICIENTS (ANOVA VALUES) FOR THE RELATIONSHIP OF SENSORY & PHYSICOCHEMICAL PARAMETERS (Y) AND REDUCED FAT CHOCOLATE BROWNIES PREPARED WITH INCREASING LEVELS OF SR USING INULIN AND REB A. (X)

Table 5a	Attribute intensity							
	Touch	Colour	Texture			Flavour		
Sample	Springiness	Crust darkness	Hard texture	Moist texture	Dense texture	Butter flavour	Chocolate flavour	Off-flavour
S75/0	0.77	0.77	-0.03*	0.99	0.94	0.88	0.85	0.91
S75/25	0.68	0.73	0.89	1.00	0.93	0.92	0.81	0.92
S75/50	-0.05*	0.82	0.87	-0.04*	0.69	0.68	0.81	0.96
S75/75	-0.01**	0.82	0.02*	-0.03*	0.94	0.84	0.60	0.71

Significance of regression coefficients*=P≤0.05, **= P≤0.01, ***= P≤0.001 (–) indicates whether the relationship is negatively correlated.

Table 5b	Hedonics					
Sample	Aroma	Appearance	Colour	Texture	Flavour	OA
S75/0	0.92	0.80	0.84	0.79	0.82	0.84
S75/25	0.84	0.93	0.47	0.81	0.73	0.75
S75/50	0.66	0.57	0.76	-0.05*	0.71	-0.04*
S75/75	-0.01**	-0.03*	-0.04*	-0.001***	-0.01**	-0.001***

Significance of regression coefficients*=P≤0.05, **= P≤0.01, ***= P≤0.001 (–) indicates whether the relationship is negatively correlated.

Table 5c	Colour		TPA			Proximate composition	
	L*		(N)			(%)	
Sample	Crust	Crumb	Hard	Gumminess	Chewiness	Sugar	Fibre
S75/0	0.46	0.60	-0.05*	0.56	0.64	0.57	0.85
S75/25	0.79	0.70	0.75	0.62	0.61	0.78	0.62
S75/50	0.78	0.61	0.83	0.47	0.44	0.71	0.87
S75/75	0.02*	0.03*	0.05*	0.57	0.58	-0.04*	0.05*

Significance of regression coefficients*=P≤0.05, **= P≤0.01, ***= P≤0.001 (–) indicates whether the relationship is negatively correlated.

Resembling results which are visually represented in Fig 3 the S75/75 sample was significantly associated with perceived hard texture and actual hard texture (N) ($p<0.05$). An increase in hardness was expected after substitution of sucrose as sucrose plays a big role in the tenderisation of baked goods, combined with this, an increased hardness in texture with the addition of inulin has been reported by O’ Brien et al. [32] and Volpini-Rapina et al. [33] in bread crumbs and orange cakes respectively. The S75/75 was found to be significantly negatively associated with perceived moist texture ($p<0.05$) as was the S75/50 sample ($p<0.05$) which means these samples were perceived as significantly dryer than any other sample. As mentioned, actual moisture content did not discriminate between samples and as a result this parameter was omitted from the PLS plot. Hardness of samples could have had a carry-over effect on perceived moisture of samples. In a study conducted by Manisha et al. [34] the addition of hydrocolloids such as xanthan gum and emulsifiers such as polysorbate-60, improved the texture of sugar replaced cakes. Hydrocolloids have shown excellent water binding capabilities and promote even crumb expansion by interfering with starch gelatinisation and starch retrogradation [34]. Emulsifiers increase volume and soft texture of cakes by promoting an even dispersion of fat which subsequently provides more areas for the expansion of gas [13]. In relation to perceived springiness of samples the S75/50 sample was significantly negatively correlated ($p<0.05$) and the S75/75 was very significantly negatively associated with this intensity attribute ($p<0.01$). Instrumental results obtained for sample springiness did not correlate with sensory results with no significant difference being found between the samples with regards to this instrumental property. The sensory results obtained for perceived springiness in this study are in agreement with results obtained from a study conducted on muffins, mentioned previously, where springiness (mm) decreased with the partial replacement of sucrose using a combination of fibres and *Reb* A. [18].

The S75/75 sample was significantly positively associated with crust and crumb lightness ($p<0.05$). A lighter crust and crumb colour as a result of SR has also been reported by Ronda et al. [36] who found that SR using polyols and other nondigestible oligosaccharides increased crust lightness of sponge cake. In a study [17] replacing sucrose with a combination of sucralose and polydextrose was found to increase crust darkness of muffins. These contradictory findings demonstrate that crust and crumb colour changes as a result of SR are dependent on the type of replacer or combination of replacers used and the products being investigated.

It is worth mentioning that no flavour intensity attributes were significantly affected by up to a level of 75% replacement of sucrose with inulin and *Reb* A.

D. Physical Properties of Chocolate Brownies

Results from physical analysis on the following treatments of Chocolate Brownies are displayed in Table 6; S0/0, S75/0, S75/25, S75/50, & S75/75. A fat replacement of 75% using pureed black beans significantly reduced sample hardness (p<0.05).

A similar result where crumb firmness decreased with the substitution of fat for carbohydrate-based fat replacers derived from gums, was reported by [37]. However, sample hardness significantly increased with increasing levels of sucrose replacement for inulin and *Reb* A. in reduced fat Chocolate Brownie samples in the range of (8.2 ± 1.21 N) for the S75/0 and (50.4 ± 1.22 N) for the S75/75 sample (p<0.05). As mentioned, hardness results obtained from instrumental analysis during sucrose replacement trials are in agreement with sensory results. Chewiness (N-mm) also significantly decreased with the substitution of 75% of fat for pureed black beans (p<0.05). Chewiness values significantly discriminated between samples containing different levels of sucrose (p<0.05) however uneven trends were observed.

TABLE 6: PHYSICAL PROPERTIES OF CHOCOLATE BROWNIE TREATMENTS

		UC0/0	S75/0	S75/25	S75/50	S75/75
TPA parameters	Hardness (N)	54.4 ± 0.44^{a}	8.2 ± 0.21^{b}	25.8 ± 0.52^{c}	30.4 ± 0.41^{d}	50.4 ± 0.52^{a}
	Gumminess (N)	-0.0 ± 0.43^{a}	1.8 ± 0.51^{b}	6.4 ± 0.67^{d}	5.6 ± 0.68^{c}	6.7 ± 0.58^{d}
	Chewiness (N-mm)	9.1 ± 0.55^{a}	0.8 ± 0.40^{b}	3.7 ± 0.60^{c}	2.7 ± 0.53^{d}	2.7 ± 0.80^{d}
	Springiness (mm)	0.5 ± 0.61^{a}	0.4 ± 0.12^{a}	0.6 ± 0.22^{a}	0.5 ± 0.22^{a}	0.4 ± 0.11^{a}
	Cohesiveness	0.3 ± 0.29^{a}	0.2 ± 0.06^{a}	0.2 ± 0.03^{a}	0.2 ± 0.02^{a}	0.2 ± 0.02^{a}
Crust colour	Lightness (L*)	39.8 ± 0.67^{a}	25.1 ± 0.50^{b}	29.2 ± 0.61^{c}	32.0 ± 0.92^{cd}	34.9 ± 0.82^{d}
	Redness (a*)	11.1 ± 0.55^{a}	11.5 ± 0.86^{a}	10.5 ± 0.86^{a}	10.6 ± 0.30^{a}	11.5 ± 0.57^{a}
	Yellowness (b*)	12.9 ± 0.64^{a}	13.0 ± 0.80^{a}	12.6 ± 0.77^{a}	12.4 ± 0.83^{a}	14.8 ± 0.70^{a}
Crumb colour	Lightness (L*)	29.0 ± 0.55^{a}	26.0 ± 0.58^{b}	27.3 ± 0.29^{bc}	30.9 ± 0.76^{c}	31.6 ± 0.52^{c}
	Redness (a*)	7.6 ± 0.81^{a}	7.1 ± 0.46^{a}	8.0 ± 0.80^{a}	8.5 ± 0.39^{a}	8.4 ± 0.93^{a}
	Yellowness (b*)	11.8 ± 0.37^{a}	11.6 ± 0.60^{a}	12.7 ± 0.55^{a}	12.4 ± 0.60^{a}	12.5 ± 0.56^{a}

[abcd] mean values (± standard deviation) in the same row bearing different superscripts are significantly different, ($p < 0.05$).

IV. CONCLUSION

The utilisation of small sugar particles allowed for fat reduction in Chocolate Brownies by up to 75% compared to the other sugar fractions investigated in this study. Small sugar particles may create the illusion of a higher fat content by retaining the perceived moist texture of reduced fat samples and therefore maintaining other key sensory properties associated with liking parameters and OA. Furthermore, samples containing small sugar particles with 25% FR were preferred to samples with no fat replacement, which further promotes the utilisation of this sugar size fraction in the application of reduced fat Chocolate Brownies.

In relation to maintaining important flavour intensity properties (Buttery, sweet), Inulin and *Reb* A. were successful up to a level of 75% replacement. This combination of sugar replacers was unsuccessful however, in maintaining colour and texture properties of samples at a level of 50% sucrose replacement. Hence liking parameters and OA were affected at this level of replacement. Perhaps the addition of hydrocolloids such as xanthan gum and emulsifying agents could improve the texture of sucrose replaced cakes using this combination of replacers. Further studies are necessary to demonstrate this.

With that said, a 25% sucrose replacement in 75% fat reduced chocolate brownie samples was achieved without affecting important sensory variables and OA. Overconsumption of these new developed products would be less likely due to increased satiety as a result of increased fibre content (3 g/100 g), which would contribute to a better caloric balance. The proposed modifications to Chocolate Brownies could be applied by industry to other cake-like products which could be a significant development in reducing the dietary intake of fat and sugar while increasing the dietary intake of fibre. Further studies are necessary to demonstrate this.

CREDIT AUTHOR STATEMENT

Aislinn Richardson: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – Original Draft, Visualization, Project administration. Andrey Tyuftin: Resources, Data Curation, Writing – Review & Editing, Visualization, Supervision, Project administration. Kieran Kilkawley: Funding acquisition. Eimear Gallagher: Funding acquisition. Maurice O' Sullivan: Validation, Resources, Supervision, Writing – Original Draft, Project administration, Funding acquisition. Joseph Kerry: Resources, Supervision, Writing – Review & Editing, Project administration, Funding acquisition.

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1. Richardson, A.M., Tyuftin, A.A., Kilcawley, K.N., Gallagher, E., O' Sullivan, M.G and Kerry, J.P. The impact of sugar particle size manipulation on the physical and sensory properties of chocolate brownies. *LWT - Food Science & Technology*, 95, 51-57, 2018.

Andrey A. Tyuftin got a Ph.D. degree in Organic Chemistry from joined DFG grant funded project between Russian Academy of Science and Dresden TU, Germany (2010). His research was focused on thiacalix[4]arenes chemistry. He also participated in a Summer research program at University of Memphis, TN, USA and was a fellow of RFBR grant for yang scientists, Moscow. After academic research Dr. Tyuftin had an industrial fellow at "Danaflex-Nano" LLC at R&D department where he was responsible for the development of high barrier food packaging materials with nano-coatings for transnational brands. Currently he is a senior postdoctoral researcher at Food Packaging group, University College Cork, Ireland where his research interests are focused on compostable, antimicrobial and smart packaging materials and novel film coating methods application with a particular involvement in new products development. His expertise is in calixarenes chemistry and packaging material science including a variety of techniques related to polymer films. Dr Tyuftin participated in different International conferences including packaging exhibitions and workshops with oral presentations in different scientific disciplines. His major publications are:

1. Tyuftin A.A., Kerry J.P. (2020). Review of surface treatment methods for polyamide films for potential application as smart packaging materials: surface structure, antimicrobial, and spectral properties. *Food packaging and shelf fife*, 24 (100475), 1-10.
2. Kerry J.P., Tyuftin A.A. (2017). Chapter 10 – Storage and Preservation of Raw Meat and Muscle-Based Food Products: IV Storage and Packaging. Book chapter in *Lawrie´s Meat Science, 8th Edition*. Ed. by Fidel Toldra. Woodhead Publishing. Elsevier. p. 718.
3. Clarke D., Tyuftin A.A., Cruz-Romero M.C., Bolton D., Fanning S., Pankaj S.K., Bueno-Ferrer C., Cullen P.J., Kerry J.P., (2017). Surface attachment of active antimicrobial coatings onto conventional plastic-based laminates and performance assessment of these materials on the storage life of vacuum packaged beef sub-primals. *Food Microbiology*, 62, 196-201.

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Eimear Gallagher got a BS, MS and PhD degree from University College Cork, Ireland. She is currently Head of Food Quality and Sensory Science Department at Teagasc Food Research Centre, Ashtown, Dublin, Ireland. Her scientific interests include wheat and flour chemistry, gluten-free science / technology. Dr. Gallagher has about 100 publications devoted to different area of Food Science. Her recent publications are:

1. Milner L., Kerry J.P., O'Sullivan M.G., Galagher E. (2020). Physical, textural and sensory characteristics of reduced sucrose cakes, incorporated with clean-label sugar-replacing alternative ingredients. *Innovative Food Science & Emerging Technologies*, 59, 102235.
2. O'Shea, N., Ktenioudaki, A., Smyth, T.P., McLoughlin, P., Doran, L., Auty, M., Arendt, E.K. and Gallagher, E. (2015). Physicochemical assessment of two fruit by-products as functional ingredients: Apple and orange pomace. *Journal of Food Engineering*, 153, 9–95.

Prof Kieran Kilcawley is a Principal Research Officer, Department of Food Quality and Sensory Science at Teagasc Food Research Centre, Moorepark, Co Cork, Ireland and an Adjunct Professor, School of Food and Nutritional Science at University College Cork, Ireland. The main focus of his research is the flavour of foods and beverages with a focus on dairy products. His main research interest is flavour chemistry, and his research covers a wide range of food and beverages. He is an editorial board member of the Journal of Dairy Research, a member of the Royal Society of Chemistry, the American Dairy Science Association, and the International Dairy Federation. He has published >80 peer reviewed publications and 14 book chapters.

Prof. Joe P. Kerry is a college lecturer and Head of Food Packaging research group, School of Food and Nutritional Sciences at University College Cork (UCC). He received his Doctorate in Microbiology at University College Galway. Dr. Kerry is also a qualified member of the Institute of Packaging. He is involved in national and international research projects both at fundamental and applied levels. Primary research interests address various aspects of food packaging, shelf-life stability, food composition and numerous aspects of food quality, particularly in relation to muscle foods and smart food packaging materials. He also has very strong links with industry and his research team assists companies in relation to many aspects of new food product development. He has over 400 publications in peer-reviewed international journals, over 200 presentations at major international conferences. His expertise includes use and manipulation of modified atmosphere packaging (MAP) systems for use with foods, use of extrusion technology for the manufacture of food products/packaging materials, applications, and sensor/new technology developments within the area of smart food packaging.

His recent publications include:

1. Kerry J.P., Tyuftin A.A. (2017). IV Storage and Packaging. Book chapter in *Lawrie´s Meat Science*, 8th Edition. Ed. by Fidel Toldra. Elsevier.
2. Malco C. Cruz-Romero, Elisa Santovito, Joseph P.Kerry, DmitriPapkovsky (2019). Oxygen Sensors for Food Packaging. Book chapter in *Reference Module in Food Science*, 1-16. Elsevier.
3. Joseph Kerry, Paul Butler (2008). Smart Packaging Technologies for Fast Moving Consumer Goods. A book. *Wiley*, p. 361.