Developing consumer acceptable biscuits enriched with Psyllium fibre

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Abstract Consumer's demand for functional food is a trend of the last decades. Nowadays, the attention is focused on the development of products with health claims on the label, which can result in value-added products for the producers companies. Cookies are popular staple foods in the human diet in many countries and are generally well accepted by consumers due to their sensory attributes, long shelf life and convenience. The incorporation of solid components on the biscuit dough, such as dietary fibre, could have serious implications on its structure and perceived texture which explains the technological limitations for the fibre incorporation. The aim of this work is to develop an enriched functional biscuit with Psyllium fibre and understand the impact of the new ingredient on physicochemical and sensorial properties of the dough and biscuits. The optimum biscuit's formulation was determined using the response surface methodology (RSM). Effects of wheat flour (48–52 %) and Psyllium (3–9 %) contents on the dimensional features (spread ratio, expansion factor), water activity, colour parameters (L^* , a^* , b^*), texture characteristics (firmness, adhesiveness, cohesiveness, springiness) and sensory properties (colour, flavour, texture, overall acceptability) of dough and biscuits were

investigated. The most representative parameters evaluated were used to develop biscuits with similar features to commercial references. An optimum formulation was obtained by incorporating 6 % Psyllium and 50 % flour. This biscuit has the enough amount of Psyllium soluble fibre recommended by Food and Drug Administration (FDA) to prevent the risk of coronary heart disease and to bear a health claim on the label.

Keywords Dietary fibre · Response surface methodology · Commercial biscuits

Introduction

The increasing consumer demand for health oriented food products has initiated the development of several bakery products enriched with new bioactive ingredients. However, reformulation of food systems to introduce new ingredients may change its structure and perceived texture, since the product is a result of the many interactions between the different components. Manohar and Rao (2002) have clearly shown the importance of rheological characteristics in influencing the quality of biscuits. Several studies have been made about the incorporation of dietary fibre in bakery products. Sudha et al. (2007) studied the influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuits quality, showing that fibres could be used for enriching the fibre contents of biscuits. The incorporation of vegetable fibre from different sources (Piteira et al. 2006) and of *Chlorella vulgaris* (Gouveia et al. 2007) and *Isochrysis galbana* microalgae biomass (Gouveia et al. 2008) was tested in biscuits similar to those used in this work.

Psyllium (alternatively known as isabgol or ispaghula) is found in the seed of plants of the *Plantago* genus, which has

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around 200 different species. From the seed coat of *Psyllium* a mucilage is obtained, by mechanical milling. The world commercial market is dominated by India, producing around 39,000 t of *Psyllium* seed every year (Bijkerk et al. 2004). Therefore, *Psyllium* consumption is very popular in India and its consumption in Europe has increased in recent years and now is also widespread in the USA.

Psyllium mucilage has a long history as a nutritional supplement due to its considerable amount of soluble and insoluble fibre being reported as a medicinally active gel forming natural polysaccharide, successfully used for the treatment of high cholesterol, diabetes, obesity in children, remediation of constipation, diarrhoea, inflammation bowel diseases and ulcerative colitis (Singh 2007). Although it's functional benefits, there are few reports about the incorporation of *Psyllium* in industrial food products (Bedard et al. 1995); in developed countries *Psyllium* consumption mainly results from the intake of capsules and other dietary preparations.

The rheology of *Psyllium* is unusual, with conformational changes occurring at low temperature and extremely strong water uptake and gelling capacities - each gram of *Psyllium* retains about 10 g of water (Kristensen and Jensen 2011). Its nutritional properties combined with gelling capacity open an important way to develop new food based on this promising natural product and the impact of this bioactive ingredient could be amplified by its incorporation on common staple foods, like biscuits. A commercial biscuit with *Psyllium* could bear the Food and Drug Administration (FDA) health claim associated with reduced risk of coronary heart disease, if the product has 1.7 g of *Psyllium* soluble fibre per reference amount usually consumed (FDA 2012). Therefore, new foods are not only fibre enriched, but also bear an FDA health claim, which is an important market advantage.

However, the incorporation of *Psyllium* in food formulations, at the levels required for health claim on the label can be a great challenge due to the very high water uptake of this polysaccharide (Cheng et al. 2009). Many physical, chemical, mechanical and enzymatic approaches have been tried to overcome this major drawback on the manufacture of the food products (Cheng et al. 2009, Yu et al. 2003), but they might produce *Psyllium* fibre containing other ingredients or intermediates with a different health impact (Yu et al. 2003).

This work is part of a project to develop new food enriched with *Psyllium* without pre-treatment, intending to contribute to an increasing intake of this dietary fibre with a FDA health claim. It was previously demonstrated in a previous work (Raymundo et al. 2014) that the maximum fibre incorporation in biscuits is about 9 % (w/w), resulting from the high water absorption capacity of *Psyllium*. In this work, the effect of composition on the dimensional features, water activity, colour, texture and sensory properties of dough and biscuits was studied. An experimental design based on the response surface methodology (RSM) was used to evaluate and quantify the

influences of the independent variables (wheat flour and *Psyllium* contents) as well as interaction effects between the variables in a specified experimental domain. To optimise the biscuit formulation in order to develop a dietary biscuit with market acceptance, a commercial standard was defined, based on the overall physicochemical properties of the similar commercial biscuits.

Materials and methods

Ingredients

Psyllium was purchased in local market (Solgar, USA), such as wheat flour (Espiga T65), sugar, margarine, baking powder and distilled water.

Psyllium was ground and only the fraction with particle size between 0.5 and 1 mm was used for all experiments.

Biscuits preparation

Considering a standard formulation established in previous work (Piteira et al. 2006), the biscuits were prepared using flour (from 48 to 52 %), 15 % sugar, 14 % of margarine and 1 % of baking powder and *Psyllium* fibre was tested at five levels – from 3.0 to 9.0 % (w/w), according to the design matrix (Table 1). The maximum level of *Psyllium* incorporation was previously tested (Raymundo et al. 2014) and it was found that *Psyllium* content over 9 % (w/w) brings technological constraints, especially induced by the rheological characteristics of dough for high levels of fibre incorporation; as result the dough fails to be moulding.

Dough was manually mixed for 5 min, sheeted with a rolling pin to a thickness of 4 mm. Then the dough was shaped and cut with a 60 mm diameter wire-cut and baked in an electrical air oven (model 23 MX-UMI, Garbin, Italy), at 180 ± 10 °C during 21 min. After cooling, biscuits were kept inside plastic bags, in sealed glass jars, at room temperature and protected from light until testing.

Physical and chemical characterisation of biscuits

Dimensions of the biscuits (diameter - D and thickness - T) were evaluated using a calliper on a set of 16 biscuits for each recipe. Spread ratio (SR) was estimated by calculating D/T values. The biscuit surface area was calculated as πr^2 . Expansion factor (EF) of biscuits was calculated according to Wang et al. (1997) through the difference between surface area of dough and biscuit after baking.

Before all the chemical determinations, biscuits were crushed to homogenise samples.

Biscuits were analysed for their moisture according to AOAC935.29 (1998) and for ash according to NP518

Table 1 Proximate composition of biscuits formulations (F1 to F12) with different Psyllium (P) and flour (F) contents, different superscript letters in the same parameter are significantly different at $p < 0.05$ level

	(%)					
	Protein	Total lipid	Moisture	Ash	Carbohydrate	
F1 (3.9 % P, 48.9 %F)	5.81±0.03 ^{b,c,d}	11.17±0.32	4.61±0.12 ^a	1.43±0.02 ^{a,b}	76.98	
F2 (3.9 % P, 51.4 %F)	6.22±0.07 ^d	11.59±0.31	4.17±0.10 ^{a,b}	1.41±0.02 ^{a,b}	76.61	
F3 (8.1 % P, 48.9 %F)	5.42±0.16 ^{b,c}	11.90±0.58	2.18±0.04 ^{c,d,g}	1.50±0.01 ^c	79.00	
F4 (8.1 % P, 51.4 %F)	5.51±0.18 ^{b,c}	11.88±0.32	1.76±0.06 ^d	1.47±0.02 ^{b,c}	79.38	
F5 (3 % P, 50 %F)	6.21±0.14 ^d	11.78±0.23	3.79±0.10 ^{a,e}	1.40±0.02 ^a	76.82	
F6 (9 % P, 50 %F)	5.48±0.19 ^{b,c}	11.93±0.52	2.16±0.13 ^{c,d}	1.47±0.01 ^{b,c}	78.96	
F7 (6 % P, 48 %F)	4.24±0.18 ^a	11.50±0.56	4.06±0.04 ^{a,f}	1.42±0.03 ^{a,b}	78.78	
F8 (6 % P, 52 %F)	5.81±0.07 ^{b,c,d}	11.62±0.19	3.08±0.10 ^{c,d,e}	1.45±0.02 ^{a,b,c}	78.04	
F9 (6 % P, 50 %F)	5.97±0.11 ^{c,d}	11.78±0.34	4.05±0.23 ^{a,b,f}	1.46±0.01 ^{a,b,c}	76.74	
F10 (6 % P, 50 % F)	5.76±0.22 ^{b,c,d}	11.84±0.04	3.75±0.03 ^{a,f,g}	1.47±0.01 ^{b,c}	77.18	
F11 (6 % P, 50 %F)	5.35±0.20 ^b	12.50±0.42	3.44±0.19 ^{b,c,d,e,f}	1.45±0.03 ^{a,b,c}	77.26	
F12 (6 % P, 50 %F)	5.53±0.21 ^{b,c}	11.84±0.10	3.26±0.06 ^{c,d,e,f}	1.44±0.02 ^{a,b}	77.93	

(1986). Total lipid analysis was carried out according to NP4168 (1991) with slight modifications and protein content was determined following ISO20483 (2006) using nitrogen conversion factor 5.70 for biscuits (Guo et al. 2008). Carbohydrate content was determined by difference to 100 % of main constituents (moisture, ash, protein and fat).

Soluble, insoluble and total dietary fibre content was determined according to AOAC 991.43 (1998) with the modifications specific for Psyllium fibre suggested by Lee et al. (1995).

Water activity (a_w) of biscuits was determined in Hygrolab equipment (Rotronic, USA) at 20 ± 0.5 °C.

All analyses were carried out at least in triplicate and expressed as the mean value and standard deviation.

Instrumental colour evaluation of the biscuits

The biscuits colour was measured using a Minolta CR-300 (Japan) tristimulus colorimeter which was calibrated using a white standard porcelain plate ($L^*96.96$; $a^*0.37$; $b^*2.10$).

The results were expressed in accordance to the CIELAB uniform colour system with reference to standard illuminate D65 (average daylight conditions) and a visual angle of 2°. The colour parameters determined were L^* which accounts for the lightness (0 % for black and 100 % white), a^* ranges

from −60 (green) to +60 (red) and b^* ranges from blue (−60) to yellow (+60). The measurements were conducted at 20 ± 1 °C under the same light conditions (50 mm² measuring area per measurement), and replicated at least 6 times.

Texture evaluation of dough and biscuits

The texture characterisation of dough and biscuits was performed using a texturometer TA-Xtplus (Stable MicroSystems, UK). The firmness (N) of the biscuits was considered as the maximum resistance to the penetration of the probe and was calculated as the height of the force peak using a cylindrical stainless steel probe of 2 mm diameter (with a load cell of 5 kg, 2 mm penetration at 0.5 mm/s crosshead speed).

The texture evaluation of the dough was performed using a texture profile analysis (TPA) in penetration mode, which consists of compressing a piece of food two times in a reciprocating motion that imitates the action of the jaw (Bourne 2002). The dough was moulded in an acrylic container (52 mm diameter and 24 mm height) and rested for 15 min before testing, using a 10 mm acrylic probe plunged 10 mm at 5 mm/s. From the force versus time texturograms, the parameters with the greater ability to discriminate the dough texture were firmness, adhesiveness, cohesiveness and springiness.

Adhesiveness is a characteristic of sticky materials and can be defined as the resistance of the material when the probe is repressing. This parameter is recorded as the negative area of the first cycle. Cohesiveness reflects how well the product withstands a second deformation relative to how it behaved under the first deformation. It is measured by the ratio between the positive area of 2nd and 1st compression cycles. Springiness is a measurement of elastic recovery, and reflects how well a product physically recovers after it has been deformed during the first compression (Bourne 2002).

These determinations were conducted at 20 ± 1 °C in a temperature controlled room and were replicated at least 8 times.

Sensory evaluation of biscuits

The sensory evaluation of the twelve biscuit formulations performed was carried out in order to observe the impact of Psyllium fibre incorporation on its sensory performance.

Fifty untrained panellists were asked to score the samples in terms of colour, taste, texture and overall acceptability using a 10 cm line scale with descriptors anchored at both ends (*dislike* and *like very much*) to describe the biscuits attributes considered.

The biscuit samples were coded with letters and served to the panellists in two consecutive days, 6 biscuits samples per day.

Experimental design

The optimisation of the texture properties of dough and biscuits and physical and sensory characteristics of the biscuits enriched with different Psyllium contents was performed according to an experimental design based on the Response Surface Methodology (RSM), using a central composite rotatable matrix (Montgomery 1991). The independent variables considered – Psyllium and flour concentrations – were tested at 5 levels (Table 1), resulting in 9 different experiments and 4 replicates of the central point. The dependent variables (responses) considered were: i) Dough: texture parameters – firmness, adhesiveness, cohesiveness and springiness; ii) Biscuits: spread ratio, expansion factor, colour parameters (L^* , a^* and b^*), water activity (a_w), firmness and sensory evaluation (flavour, colour, texture and overall acceptability).

Target definition for optimisation

To optimise the biscuits formulation in order to develop a biscuit enriched with Psyllium fibre similar to commercial products, 11 reference biscuits with high fibre content from a wheat source (whole wheat, wheat or wheat bran) were chosen to be used as target. Proximal composition and energy value of samples were taken from the product's package

information. Texture, water activity and colour parameters were evaluated according to the methods described earlier. For each parameter, a target and the range of variation were defined. Then, the values obtained for sample biscuits were plotted against the target.

Statistical analysis

Experimental data were analysed by means of one-factor analysis of variance (one-way ANOVA or Kruskal-Wallis test). When the analysis of variance indicated differences among means, a Scheffé test or pairwise comparisons were performed to differentiate means with 95 % confidence ($p=0.05$). All statistical treatments were performed using SPSS Statistics (v.20, IBMSPSS Statistics, New York, USA).

RSM analysis was performed using the software Statistica (version 10.0, StatSoft Inc., USA).

Results and discussion

Physical and chemical characterisation of biscuits

In Fig. 1 are depicted the spread ratio (width/thickness) and expansion factor (relation between biscuit and dough volumes) of the matrix formulations.

It was observed that the spread ratio (SR) increases significantly ($p<0.05$) with the incorporation of Psyllium from 3 % (F5: SR=6.1) to 9 % (F6: SR=6.8), mainly due to variations in biscuit thickness which could result from the higher hydration capacity of Psyllium. This parameter is sensitive to the mixing time and the baking process (Cronin and Preis 2000). After baking, biscuits usually present a higher diameter due to low gluten content, which results in the formation of a fragile net instead of a viscoelastic one (Zoulias et al. 2002). In this case, the diameter of all biscuits decreased after baking, probably due to Psyllium ability to form gel (Fischer et al. 2004) and thus contributing to diminish this tendency. These findings are supported by Sudha et al. (2007) study in which the incorporation of different sources of bran showed opposite relations with spread ratio.

Spread ratio and expansion factor present a strong negative correlation (-0.936), indicating that dough with higher Psyllium incorporation lead to the production of smaller biscuits. This is probably due to the lower gluten content of these biscuits and lower level of expansion of Psyllium fibre, not allowing the biscuits to expand properly.

The proximate composition of biscuit matrix formulations is given in Table 1.

There are no significant differences ($p<0.05$) among the lipid values of all the biscuits formulations (11.17–12.50 %). Since the lipid content of both Psyllium (P: 0.95 %) and flour

Fig. 1 Spread ratio and expansion factor of biscuits formulations (F1 to F12), different superscript letters in the same parameter are significantly different at the $p < 0.05$ level

(F: 1.44 %) is similar (Raymundo et al. 2014), the replacement of wheat flour with Psyllium does not significantly affects the lipid content of the final product.

Small but statistically significant differences ($p < 0.05$) were obtained among the ash contents of the formulations. Increasing the Psyllium content resulted in increased ash content of the biscuits due to its higher value in Psyllium (2.25 %) than in wheat flour (0.50 %) (Raymundo et al. 2014). Similar results were found by Uysal et al. (2007) with the incorporation of apple and lemon fibre in cookies.

A decrease in protein content with Psyllium incorporation (6.22 to 4.24 %) was also observed, resulting from the highest protein level of wheat flour (9.0 %) compared with Psyllium (1.4 %) (Raymundo et al. 2014). As gluten formed during mixing is responsible for the viscoelastic properties of dough and a major factor influencing biscuit quality (Manohar and Rao 2002), these results will be taken into account for the selection of the optimum formulation.

Significant differences ($p < 0.05$) in the moisture content of the developed biscuits were observed. This parameter decreased with an increase of Psyllium incorporation, although flour may also play a role in this result. As determined in previous work (Raymundo et al. 2014), the moisture content in flour and Psyllium is similar, so the replacement of flour for Psyllium could be explained by the high water holding capacity of Psyllium (more than 16 g/g of water). Opposite findings were encountered by Uysal et al. (2007) with the incorporation of apple and lemon fibre in cookies.

Although biscuits are a relatively dry product in terms of moisture, its water activity (Fig. 2) is crucial to predict both the stability and safety of this product in terms of microbial growth. The lowest a_w at which the vast majority of food spoilage occurs is 0.90, although some moulds and yeasts grow above 0.61 (Fontana 2001).

A significant reduction in water activity with high levels of Psyllium was observed, which could lead to a greater shelf life of the biscuits, although it was reported (Arimi et al. 2010) that loss of crispiness is related to an increase of a_w . These results are probably due to the interaction between water and hydroxyl groups of the polysaccharides present in the fibre macromolecules, through hydrogen bonds (Dikeman and Fahey 2006) mainly established by arabinose and xylose molecules in the case of Psyllium (Temudo et al. 2008).

The insoluble and soluble fibre fractions of the nine formulations studied are represented in Fig. 3. Based in numerous studies reporting health beneficial effects of Psyllium consumption (Garcia et al. 2005, Marlett and Fischer 2003, Olson et al. 1997, Sudha et al. 2007), the FDA approved health claim for its soluble fibre related to lowering risk of coronary heart disease effects (FDA, 2012).

Formulations F3, F4 and F6 presented higher significant levels of insoluble fibre content (Fig. 3), mainly due to the higher level of Psyllium incorporated (8.1 and 9 % Psyllium). Although the soluble fibre constitutes the main fraction in Psyllium, only biscuits prepared with 6 % Psyllium (F7, F8 and F9) showed higher content of soluble fibre. This result may be explained by the fibre content of wheat flour (9.9 % soluble fibre and 6.5 % insoluble fibre (Raymundo et al. 2014), the main ingredient of biscuits.

Instrumental colour evaluation of biscuits

Colour characteristics of ingredients, mainly lightness, play an important role in bakery products, strongly influencing consumer acceptability (Angioloni and Collar 2011). It is also important for the identification of product's quality, flavour expectation and commercial value.

No significant differences ($p < 0.05$) are observed for the colour parameters of all the biscuit formulations tested. These

Fig. 2 Water activity of biscuits formulations (F1 to F12), different superscript letters in the same parameter are significantly different at the $p<0.05$ level

results may be due to the protein present in wheat flour, responsible for the effects of caramelisation and Maillard browning reactions between the protein and the sugar present, as reported by Gallagher et al. (2003). This may also be due to the resemblance of colour between Psyllium and baked wheat flour.

It was also observed that biscuits with higher levels of Psyllium showed a wrinkled surface, as previously described by Sudha et al. (2007) in relation to the addition of bran responsible for the reduction of surface smoothness.

Texture evaluation of dough and biscuits

The evaluation of dough and biscuits texture parameters is depicted in Figs. 4 and 5.

For the same flour content, significant differences ($p<0.05$) among the firmness of the formulations with different Psyllium contents were observed. As an example, for biscuits with 50 % flour it is observed that firmness increases with the incorporation of Psyllium (3 % P - 8.05 N; 9 % P - 20.9 N). This results from the presence of xylose and arabinose from Psyllium that promote interaction between polysaccharides and proteins, strengthening the biscuit structure

(Sudha et al. 2007). It is also observed that the biscuits firmness increased with protein (from flour) content (for ex: 8.1 % Psyllium - 20.7 N for 48.9 % Flour compared to 28.2 N for 51.4 % Flour), as described earlier by Fustier et al. (2009).

The same relations were found for dough firmness, which corroborates Sudha et al. (2007) findings that in the presence of bran from different sources the dough becomes harder originating harder biscuits. As firmness of the biscuit depends upon the characteristics of the initial dough, a relationship between the firmness of dough and biscuits was established, both in terms of the strength of the relationship and the direction (Pallant 2001). For this purpose, a correlation between both variables was calculated with a coefficient of 0.765, indicating a good relation between the firmness of dough and biscuit.

Texture profile analysis of the dough (Fig. 5) did not allow to discriminate the formulations based on the analysed parameters, since significant differences ($p<0.05$) among the biscuits was not observed. These results are the opposite to those reported by Saha et al. (2011) which found that wheat flour increased the cohesiveness of the dough due to the plasticity of gluten, and

Fig. 3 Fibre content (soluble and insoluble) of the biscuits formulations (F1 to F9), different superscript letters in the same parameter are significantly different at the $p<0.05$ level

Fig. 4 Firmness of dough and biscuit matrix formulations, different superscript letters in the same parameter are significantly different at the $p<0.05$ level

that the adhesiveness was slightly lower at lower wheat flour level.

Sensory evaluation of biscuits

The results of the sensory evaluation are summarised in Table 2.

The biscuits F5 were the least preferred by the consumer's panel, scoring a statistical significantly lower value ($p<0.05$) in the overall acceptability. In terms of colour slightly differences were observed, but F8 got the highest score. F2 scored the highest overall acceptability value, but only statistically different from F5. The score of texture reduced significantly from 6.74 to 5.12, being F7 the favourite. Higher texture score are associated with better eating quality of biscuits (Manohar and Rao 2002), with the best scores in flavour and texture. The F7 formulation was also the preferred in terms of flavour, but with no statistical significance.

These results are not in accordance with ones of Sudha et al. (2007), who reported low scores with increase level of bran in biscuits.

Optimisation of the texture properties of dough and biscuits and physical and sensory properties of the biscuits

The response surface methodology has been widely used to minimize the number of trials while studying ingredients interactions and quality characteristics of biscuits (Gallagher et al. 2003, Moraes et al. 2010), cakes (Gan et al. 2007) and vegetable gelled desserts (Nunes et al. 2006).

The response surface plots with statistical significant ($p<0.05$) are depicted in Fig. 6a to e.

The other parameters analysed - adhesiveness, cohesiveness, springiness, spread ratio, expansion factor, colour parameters (L^* , a^* and b^*), and sensory flavour and overall acceptability - did not show statistical correlation with the

Fig. 5 Texture parameters (adhesiveness, springiness and cohesiveness) for dough formulations F1 to F12, different superscript letters in the same parameter are significantly different at the $p<0.05$ level

Table 2 Sensory analysis of the biscuit formulations (F1 to F12) with different Psyllium (P) and wheat flour (F) contents, different superscript letters in the same parameter are significantly different at $p<0.05$ level, bold values indicate the consumer's preferences for each parameter

Formulation	Sensory parameters			
	Colour	Flavour	Texture	Overall acceptability
F1 (3.9 % P, 48.9 %F)	6.9±1.7	5.9±1.8	5.5±2.1 ^{a,c}	6.0±2.1 ^{x,z}
F2 (3.9 % P, 51.4 %F)	6.9±1.6	6.5±1.6	6.6±1.8 ^{a,c}	6.9±1.5^x
F3 (8.1 % P, 48.9 %F)	6.8±1.9	6.4±1.9	6.7±1.6 ^{b,c}	6.7±1.8 ^{x,z}
F4 (8.1 % P, 51.4 %F)	7.3±1.4	6.3±1.9	6.0±2.2 ^{a,c}	6.5±1.8 ^{x,z}
F5 (3 % P, 50 %F)	6.6±1.9	5.2±2.4	5.1±2.1^a	5.8±2.1^y
F6 (9 % P, 50 %F)	6.9±1.7	6.2±2.0	6.4±2.1 ^{a,c}	6.6±1.7 ^{x,z}
F7 (6 % P, 48 %F)	6.6±1.8	6.7±1.9	6.7±1.9^{b,c}	6.6±1.8 ^{x,z}
F8 (6 % P, 52 %F)	7.4±1.4	6.5±2.0	6.7±1.5 ^{b,c}	6.8±1.6 ^{x,z}
F9 (6 % P, 50 %F)	6.8±1.5	6.4±1.7	6.2±2.2 ^{a,c}	6.9±1.7 ^{x,z}
F10 (6 % P, 50 %F)	6.8±1.7	6.4±1.5	5.7±1.7 ^{a,c}	6.1±1.5 ^{x,z}
F11 (6 % P, 50 %F)	6.7±1.9	6.2±1.9	5.9±1.9 ^{a,c}	6.7±1.5 ^{x,z}
F12 (6 % P, 50 %F)	6.8±1.7	6.1±1.6	6.1±1.8 ^{a,c}	6.0±1.5 ^{x,z}

Psyllium and flour concentrations, since the coefficient of determination (R^2), which indicated the fitting of the model to the variable responses, is lower than 0.8 (Gan et al. 2007).

A good reproducibility of this method was observed, as the 4 central points of the matrix presented similar values for all the responses studied.

Fig. 6 Response surface plots of the effects of flour and Psyllium. (a) on the dough firmness; (b) on the firmness of biscuits; (c) on the a_w of biscuits; (d) on the sensory score on texture of biscuits; (e) on the sensory score on colour of biscuits

Fig. 7 Physicochemical properties of biscuits (dotted lines represent the maximum and minimum values of the reference biscuits). **(a)** Firmness of biscuit formulations F1 to F12; **(b)** Water activity of biscuit formulations

F1 to F12; **(c)** Total fibre content of biscuit formulations F1 to F12. (* the s.d. was calculated from the s.d. of formulations F9 to F12)

Dough characterisation

The dough texture can be expressed in terms of the statistical equation obtained from the multiple regression of the results (Eq. 1). This equation, valid in the experimental domain studied, reveals that dough firmness has a negative linear and a positive quadratic dependence with Psyllium content, and a positive linear dependence with wheat flour content (Fig. 6a).

$$\text{Dough Firmness(N)} = -9.86 - 0.20[P] + 0.05[P]^2 + 0.21[F] \quad (R^2 = 0.9835, R^2_{\text{adjust}} = 0.9773) \quad (1)$$

Biscuits characterisation

The statistical correlations obtained from the multiple regressions of the results, concerning the analysis of biscuits, can be expressed in terms of the Eqs. 2, 3, 4 and 5, where SA stands for sensory analysis.

$$\text{Firmness(N)} = -189.31 + 10.18[P] - 0.61[P]^2 + 3.40[F] \quad (R^2 = 0.8883, R^2_{\text{adjust}} = 0.8464) \quad (2)$$

$$\text{Water activity}(a_w) = -3.82 - 0.08[P] - 0.06[F] \quad (R^2 = 0.9257, R^2_{\text{adjust}} = 0.9092) \quad (3)$$

$$\text{Texture(SA)} = -21.70 + 8.88[P] + 0.01[F]^2 - 0.17[P][F] \quad (R^2 = 0.8854, R^2_{\text{adjust}} = 0.8424) \quad (4)$$

$$\text{Colour(SA)} = 163.62 - 2.62[P] - 6.11[F] + 0.06[F]^2 + 0.05[P][F] \quad (R^2 = 0.8635, R^2_{\text{adjust}} = 0.7856) \quad (5)$$

It can be observed that the firmness depended on both Psyllium and flour contents, although the fibre plays a more determinant role on the variation of this parameter, showing a quadratic effect.

The water activity was negatively dependent on both Psyllium and flour contents, meaning that increasing one or both ingredients will lead to a decrease of a_w . This could be beneficial for controlling the microbial growth of the product.

Sensory scores on texture and colour of the biscuits also depend on both variables, but at different levels. Increasing Psyllium content will lead to an increase in the sensory score of texture and a less important decrease in colour score.

Optimisation of biscuit formulation

The optimum biscuit formulation was selected considering the comparison with commercial cookies enriched in fibre, using

the most representative physicochemical properties of the developed biscuits (Fig. 7a to c), namely firmness, a_w , fibre and lipid contents, energy value and sensory analysis. This kind of approach has already been done in vegetable desserts (Nunes et al. 2006) and cassava cake (Gan et al. 2007).

Based on the firmness values it is possible to conclude that F1, F2, F5 and F7 met the requirements of the commercial biscuits. These biscuits had the lower Psyllium incorporations (3.0 and 3.9 %) except for F7. This is probably due to the fact that the firmness of biscuits depends on both Psyllium and flour concentrations (Fig. 6a). In this case, F7 has 6 %P but only 48 %F.

Crossing these results with the ones from the consumer's panel (Table 2), it is concluded that F7 was the preferred biscuit in terms of texture.

Despite only F3, F4 and F6 presented a_w values within the range of variation obtained for the commercial biscuits, the other formulations had a_w values lower than the minimum limit for microbial growth. These biscuits were prepared with high Psyllium contents (8.1 and 9.0 %), and presented much greater firmness values than the references (Fig. 7a). So, although a_w is an important parameter for predicting the quality of food products, the selection of the optimum formulation will not take into account these results.

In terms of colour, all formulations are within the range of variation of commercial biscuits ($58.21 \% < L^* < 100 \%$; $1.42 < a^* < 12.56$; $3.99 < b^* < 22.87$). As shown in Table 2, through sensory analysis, the consumers preferred F8 colour, although with no statistical significance.

All Psyllium biscuits formulations presented a higher fibre content than all the commercial references used (Fig. 7). This result presents a good alternative to other enriched fibre biscuits, namely containing 20 % barley bran (Sudha et al. 2007) which translates in only 9.3 % total dietary fibre (TDF), much lower than the 22.6 % TDF of the chosen biscuit (F7).

For nutritional purposes, it is also important to access the biscuits developed in terms of lipids and energy value (data not shown). The lipid values of Psyllium biscuits are close to the lower limit of the range for the references (11.30–21.30 %). This fact along with the similarity in energy value of both set of biscuits provides an opportunity to develop a fibre enriched product with low fat content. It is also important to highlight that in terms of flavour the consumers were very receptive to the product, being F7 the favourite biscuit, although with no statistical significance.

Given the wide variety of this type of products in the market, a new functional biscuit must show a strong and important added value in order to gain place in this competitive sector. With this in mind, the proposed biscuit is not only fibre enriched, but also enriched with Psyllium fibre, which bears an FDA health claim associated with reduced risk of coronary heart disease.

The optimum formulation of biscuit enriched with Psyllium fibre was obtained with 6 % Psyllium and 48 % Flour (formulation F7). To bear the FDA health claim, the product should have 1.7 g of Psyllium soluble fibre per reference amount usually consumed (FDA 2012). On a daily basis, people generally consume 3 biscuits (± 14 g/biscuit). Considering the formulation selected (F7), the soluble fibre from this source would allow this product to bear a health allegation on the package information.

Conclusions

The production of biscuits enriched with Psyllium husks can be considered as an alternative way to include this health promoter fibre in human nutrition.

The optimisation of the formulation resulted in 6 % Psyllium incorporation, sufficient to produce a consumer acceptable biscuit enriched with fibre, which can use a health claim on the label.

However, it is necessary to improve technological developments that enable the increasing incorporation levels and the development of other staple foods enriched with Psyllium fibre.

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