1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads.

Sandra Pérez-Quirce¹, Concha Collar², Felicidad Ronda^{1*}

(1) Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

(2) Cereal Group, Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos, IATA-CSIC, Avda. Catedrático Agustín Escardino 7, 46980 Paterna, Spain.

*Corresponding author: Felicidad Ronda. Tel : 34 979108339 ; E-mail: fronda@iaf.uva.es

Abstract

The impact of associated viscous dietary fibers (hydroxypropylmethylcellulose semi-firm –SFE- and weak –NE- gel forming, and barley β -glucan, BBG) incorporated at different amounts (1.6–7.5%, flour basis) into gluten-free rice-based dough formulations, on the breadmaking performance and staling behaviour of hydrated (70–110%, flour basis) fibre-flour composite blends has been investigated.

Single BBG addition fails to mimic gluten visco-elasticity properly, but simultaneous incorporation of either SFE or NE contribute to bread improvement in terms of bigger volume and smoother crumb. ~~3.3-g of BBG (70% purity) and 104mL of water addition to 100-g rice flour provided sensorially accepted breads (7.6/109) with a theoretical β -glucan content of 1.24-g/100-g GF bread that would allow a daily β -glucan intake of 3 g with provided a bread consumption of 240g/day. A daily intake of 240-g of BBG-enriched GF bread (four servings) is high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a reduced blood cholesterol level. Complementary tests should be carried out to test know the amount and molecular weight of β -glucan in the final bread before assuring the nutritional benefit of this addition.~~

Keywords: Gluten-free; Hydrocolloids; β -glucan; bread quality

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

1. Introduction

The increased consumer demand for healthy foods has driven to address concerted efforts from both research and industry to develop breads that combine properly health benefits with good physico-chemical and sensory properties. This goal is specially challenging in gluten-free (GF) breadmaking where the lack of gluten biopolymer seriously constrains dough visco-elastic character, leads to a failure in carbon dioxide entrapment, and hence deteriorates the techno-functional quality of resulting breads. In addition, a poor nutritional balance often characterises the multi-ingredient GF matrices (Thomson 2009). GF baked goods are often low in fibre, both soluble and insoluble; consequently its enrichment with dietary fibre seems to be necessary (Sabanis et al. 2009).

The natural, synthetic and biotechnological hydrocolloids, because of their high water-binding capacity and their structure-creating behaviour, are mostly used in the different recipes for replacing the gluten network and its functionality (Houben, Hoechstoeetter, and Becker 2012). Water availability plays a crucial role in the functionality of hydrocolloids by binding to the macromolecules in three different ways: via hydrogen bounds, embedded in inter- or intramolecular openings or immobilized by structuring (Anton 2008). The modified cellulose derivative hydroxypropyl-methyl-cellulose - HPMC- (linear and neutral polymer) has, because of its hydrophilic character, a high water-binding capacity and also has, in its structure, hydrophobic methyl and hydrophilic hydroxypropyl groups located, which makes HPMC an interface activity in the dough system during the resting period promoting dispersion and preventing coalescence of the gas bubbles. HPMC can create a reversible, heat-set gel network (Haque et al 1993) that leads to an increase in dough viscosity and stabilization of the

boundaries of the expanding gas cells. During baking, the gas-binding capacity is increased and higher volume can be achieved (Bell 1990; Collar et al 1999).

The positive effects of isolates of cereal β -glucans have been recently reviewed by Wood (2010), with most of the data deriving from studies with oat β -glucans, followed by barley and rye (Kinner et al 2011). The European Food Safety Authority (EFSA) has recently approved health claims for foods that contribute to the diet 3 g per day of β -glucan (BG) from oat or barley for its ability to reduce the LDL-cholesterol level in blood, and for foods that provide at least 4 g / 30 g carbohydrate, for reducing the postprandial glycaemic response (EFSA 2011). Isolates of cereal β -glucan are hydrocolloids with thickening properties (Lazaridou et al 2007) that could replace or supplement the action of HPMC when added in appropriate amounts, besides increasing nutritional value of GF bread in terms of dietary fiber content with proven health promoting effects. It is also stressed that high concentration of β -glucan decreases the water availability for the protein network and thus impairs the baking properties of wheat breads (Gill et al 2002). Molecular mass and structure, chain length, bonds and chemical modification, added doses, raw materials and process parameters used -pH value, temperature, shearing, ionic bonds and the attendance of ions- account for some factors determining the significance of associated hydrocolloids in bread performance (Houben, Hoechstoeffer, and Becker 2012).

Previous studies have shown a great difference in the effect of β -glucan and HPMC of different gel strengths on gluten-free rice dough viscoelastic behaviour (Collar et al 1999; Collar, Santos, and Rosell 2005; Collar, Santos, and Rosell 2007; Ronda et al 2013). The water competition of the fibre macromolecules expliciting different water binding and gelling abilities resulted in additive, synergistic and/or antagonistic effects on major rheological features. The present study aims to establish the effect of -viscous

1
2
3
4
5
6 90 dietary fibres blends at different hydration levels on GF bread quality and ~~its~~-staling
7
8 91 ~~keepability~~. A correlation study between dough and bread properties was carried out to
9
10 92 know the relationships between rheological performance and physico-chemical and
11
12 93 sensory pattern of β -glucan-enriched GF breads.

14
15 94 **2. Material and methods**

16
17 95 2.1 Materials

18
19 96 Rice flour (12.5% moisture, 0.46 % ash, 7.5% protein, 0.49 % fat and 79.1 % starch;
20
21 97 ~~particle size distribution: 6% > 150 μ m, 150 μ m > 63.2% > 100 μ m, 30.8% < 100 μ m)~~
22
23 98 was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower
24
25 99 oil were purchased from the local market. Two types of hydroxypropylmethylcellulose
26
27 100 (HPMC) (E464) from Shin-Etsu Chemical Co Ltd. (Japan) were used: HPMC SFE-
28
29 101 4000 (27 – 30 % methoxyl content, 4.0 – 7.5 % hydroxypropoxyl content) coded SFE
30
31 102 and HPMC NE-4000 (19 – 24 % methoxyl content, 4.0 – 12 % hydroxypropoxyl
32
33 103 content) coded NE. According to manufacturer’s application notes, both types of HPMC
34
35 104 (10 % moisture content) develop the same apparent viscosity (4300 ± 1300 cP) in 2%
36
37 105 aqueous solution at 20°C, but different gel strength after thermal treatment. HPMC-SFE
38
39 106 ~~4000~~-forms a semi-firm gel (gelation temperature 58-63°C) while HPMC-NE ~~4000~~
40
41 107 forms a weak and sticky gel (gelation temperature 61-65°C). (1-3)(1-4) β -glucan (BBG)
42
43 108 was obtained from barley, Barliv™, and supplied as free sample by Cargill (Barcelona,
44
45 109 Spain). The characteristics of BBG were: 6 % moisture, 2.2 % soluble protein, 2.6 %
46
47 110 ash and 0.9 % fat (commercial data); 70% purity (Megazyme® kit); 5.6 % starch
48
49 111 (Megazyme® kit); 140 kDa molecular weight (size exclusion HPLC). BBG extract was
50
51 112 analysed for gluten content and a concentration under the detection limit was obtained
52
53 113 (< 6.2 mg/kg of ~~g~~Gluten) using the ELISA test based on the R5 antibody.

54
55 114 2.2. Dough preparation and breadmaking

1
2
3
4
5
6 115 A straight dough process was performed using the following formula on a 100 g rice
7
8 116 flour basis: 6% oil, 5% sucrose, 2 % salt, 3% dried yeast, and 70% water. Combinations
9
10 117 of fibres according to a Draper-Lin small composite design for sampling (Draper and
11
12 118 Lin 1990) were added to the basic formula at different hydration levels (Table 1).
13
14 119 Design factors (quantitative independent factors) tested at five levels (-1.4142, -1, 0, 1,
15
16 120 1.4142), included SFE (from 0.10 to 2.50 g/100 g flour basis), NE (from 0.10 to 2.50 g
17
18 121 /100 g flour basis), BBG (from 0.10 to 3.90 g/100 g flour basis), and WATER (from 0
19
20 122 to 40 mL extra water with respect to 70mL/100 g flour basis, that was the minimum
21
22 123 amount added).—The model resulted in 19 different combinations of fibre-enriched
23
24 124 hydrated rice-based doughs, including three central point replicates. These replicates
25
26 125 were made in order to know the repeatability and accuracy of results. GF dough-making
27
28 126 was achieved by blending first solid ingredients in a kitchen-aid professional mixer
29
30 127 (KPM5) for 10 s at speed 2. Then, liquid ingredients (oil and water at 20 ± 2 °C) were
31
32 128 added and mixed for 5 min at speed 6. The dough, 200 g, was placed into an aluminium
33
34 129 oil coated pan and was proofed at 30°C and ~~(90 ± 5)~~ % relative moisture for 40 min.
35
36 130 Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190°C for 40
37
38 131 min. After baking, breads were removed from the pan and left for one hour at room
39
40 132 temperature before analysis. ~~To study the effect on staling, breads were stored for 0, 1,~~
41
42 133 ~~2, 5, 7 and 9 days at (4 ± 2) °C.~~ To study the effect on staling, breads were stored in
43
44 134 hermetic polypropylene bags for 0, 1, 2, 5, 7 and 9 days at (4 ± 2) °C. Seven breads
45
46 135 were made in each per run (one batch).

136 137 2.3. Evaluation of bread quality

1
2
3
4
5
6 138 Bread volume was determined in duplicate using a volume analyzer BVM-L370 TexVol
7
8 139 Instruments (Viken, Sweden). The bread was weighed immediately after removal from
9
10 140 the pan once cooled. A digital calibre was used to measure bread height and width.
11
12 141 Crumb texture was determined in triplicate with a TA-XT2 texture analyser (Stable
13
14 142 Microsystems, Surrey, UK) provided with the software “Texture Expert”. An
15
16 143 Aluminium 20 mm diameter cylindrical probe was used in a “Texture Profile Analysis”
17
18 144 double compression test (TPA) to penetrate to 50% depth, at 1 mm/s speed test, with a
19
20 145 30 s delay between first and second compression (Collar, Bollain, and Angioloni 2005).
21
22 146 Hardness (N), chewiness (N), cohesiveness, springiness and resilience were calculated
23
24 147 from the TPA graphic. Analysis was carried out at (20 ± 2) °C for bread slices of 20 mm
25
26 148 thickness taken from the centre of the loaf.
27
28 149 Colour was measured with a Minolta spectrophotometer CN-508i (Minolta, Co.LTD,
29
30 150 Japan). Results were obtained in the CIE L*a*b* coordinates using the D65 standard
31
32 151 illuminant, and the 2° standard observer (CIE 1931). The hue (h) and the chroma (C*)
33
34 152 were calculated from them with the equations $h = \text{atan}(b^*/a^*)$ and $C^* = ((a^*)^2 + (b^*)^2)^{1/2}$
35
36 153 (Ronda et al 2005). L* ranges from 0 (black) to 100 (white). The hue scale extends from
37
38 154 0° (red), 90° (yellow), 180° (green) to 270° (blue). The chroma informs about the purity
39
40 155 of the colour: a near zero C* value corresponds to a colour of low purity, near grey. On
41
42 156 the opposite high C* values mean colours of high purity near the pure spectral colours.
43
44 157 Colour determinations were made 5x5 times: bread crumb and crust colours were
45
46 158 checked at five different points on each bread and every point was measured five times.
47
48 159 2.4 Sensory analysis
49
50 160 Sensory analysis was performed by a panel of ten semi-trained judges (four males and
51
52 161 six females aged 25–52), who scored the size and the uniformity of cells (crumb grain),
53
54 162 the softness and chewiness of the crumb, the crumbliness of the crust, the taste and

~~flavour (intensity and type), and the overall acceptability.~~ Ratings were given to breads wrapped in plastic bags and stored at room temperature for 24 hours. A semi-structured scale from 1 ~~(very little)~~ to ~~109 (very much)~~ was used. The attributes tested were: crumb grain (1=very large and inhomogeneous cells; 10= very small and homogeneous cells), crumb softness (1= very hard; 10= very soft), crumb chewiness (1=very rubbery, requiring many bites to swallow; 10= very little rubbery), crust crumbliness (1= very soft and annealed crust; 10=very crunchy crust) taste and flavour intensity (1: very little; 10: very much). Additionally, ~~A~~an hedonic, ~~-overall acceptability~~ test was also included in the sensory evaluation with an scale from 1 (dislike very much) to 10 (like very much).

2.5. Statistical analyses

Multivariate statistical analysis of data (non-linear regression, stepwise regression analysis, and Pearson correlation analysis) was performed by using Statgraphics Centurion v.6 program (Bitstream, Cambridge, MN, USA).

3 Results and discussion

~~Morpho-geometrical features, texture characteristics and sensory scores were measured to assess the quality and staling behaviour of fibre enriched GF breads made according to a Draper Lin design. Retrieved instrumental physical parameters and sensory results were analysed for dependence on dough hydration and on viscous dietary fibres, and for correlations between dough and bread parameters (Table 5 and 6).~~

3.1 Effect on physical properties of fresh breads

Analytical data on bread characteristics ~~(Table 2)~~ were fitted to multiple regression equations using added principles (SFE, NE, BBG, WATER) as independent factors in order to estimate response surfaces of dependent analytical variables. Significant

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

coefficients (95% confidence interval) obtained from the stepwise regression fitting model are included in Table 32.

Specific volume and height/width rate

Loaf specific volume of the breads, that varied between 1.4 and 5.1 mL/g, and the loaf height/width ratio, that ranged 0.28 – 0.87, exhibited similar trends, as could be expected in pan breads ~~where the width comes mainly determined by the mould size.~~

The multiple regression equations obtained for height/width and specific volume explained the 99% and 91% of their variability, respectively (Table 42). Specific volume increased with WATER, SFE and BBG addition until a maximum ~~(Fig.1a and Fig.1b).~~. The positive coefficients of the linear terms and the negative coefficients of the quadratic ones of these three factors account for this evolution (Table 24). It should be noticed the non significant individual effect of NE ($p>0.05$) on both the height and the specific volume of breads, were probably masked by the greater effect of the remaining design factors. ~~The comparison by pairs of the experimental results obtained for each run (runs 1, 5, 10), allowed to confirm a significant increase ($p<0.05$) in the specific volume for increasing SFE amounts, provided remaining factors keep constant (data not shown).~~ A significant positive interaction SFE*NE was observed, which means that NE enhanced the individual SFE effect on bread size. The effect of SFE on specific volume did not showed a significant dependence on dough water content, being 1.6% the dose that led to the maximum size of the bread ~~(Fig.1.a)~~. From the multivariate regression equation the individual addition of 1.6% SFE to the dough with the minimum water content tested (70%, equivalent to 0 level in the design) would nearly double the initial volume of the bread, ~~passing from 1.4 mL/g to 2.7 mL/g~~. For this SFE dose, a water increase from 70% to 90% would lead to an additional specific volume increase by 37%. 90% of dough hydration ~~(equivalent to 20 level in the design)~~ led to the

maximum bread specific volume, only dependent on BBG dose. The positive interaction BBG*WATER (Table 42) indicates that water favors the effect of BBG on specific volume. ~~However optimal water content of dough was not significantly dependent on SFE and NE.~~ This fact establishes an important difference between the HPMC and BBG action on GF breads: BBG requires an important additional amount of water to show a beneficial effect on bread volume while HPMC acts even in adverse conditions of low dough hydration showing an effect little dependent on dough water content. ~~The effect of BBG on the bread specific volume is shown in Fig 1b.~~ The individual addition of BBG to dough (70% water, ~~flour basis~~) decreased markedly the height and the volume of GF breads ~~(see also the negative coefficients of the quadratic terms)~~. The same effect was observed in wheat breads by Brennan & Cleary (2007) when β -glucan content varied from 2.5% to 5%. Some authors (Cavallero et al 2002; Gill et al 2002) relate the above mentioned effect to the high water binding capacity of β -glucan that restricts available water for the development of gluten network. The same reason might explain BBG effect on GF breads. ~~(Ronda et al. 2013).~~ Doughs with too low disposable water are probably too consistent to get a certain development during proofing and subsequent baking ~~(Ronda et al. 2013)~~. BBG added to reduced-water doughs is unable of establishing cross-links or entanglements in the dough and, thus, dough structure cannot be developed. It was previously shown that BBG was unable to decrease the elastic compliance, J_0 , at dough water content of 70% as HPMC did (Ronda et al 2013). But, in these conditions, it conferred the major effects on dough G' modulus. So that, BBG addition to doughs only increased dough consistency, which made dough development even more difficult, leading finally to a lower bread volume. Simultaneous addition of water and BBG counteracted the single BBG effect ~~through the significant positive coefficient of the interaction BBG*Water (Table 4),~~ leading to an increase of 34% in

Formatted: English (U.K.)

1
2
3
4
5
6 237 bread volume compared to the maximum value obtained in BBG absence. In a previous
7
8 238 work (Ronda et al. 2013), a significant BBG*Water negative interaction on the elastic
9
10 239 modulus G' was found -for the same GF doughs, -allowing to relate the increase in
11
12 240 bread volume with a decrease in dough consistency. The additional amount of water
13
14 241 required to get the maximum volume in bread increased at a rate of 5 - 6 % per 1%
15
16 242 increase of BBG in dough formulation (flour basis in both cases): the dough hydration
17
18 243 needed for 2% BBG would be 101%, and for 3.5% BBG it would be 109 %. However,
19
20 244 the model predicts that without HPMC, even with the optimal dose of water, BBG
21
22 245 addition above 2.5% would start to decrease specific volume attaining a reduction of
23
24 246 24% at the maximum dose tested (3.9%). Lazaridou et al. (2007) also observed a
25
26 247 maximum on bread specific volume with water, and obtained an additional increase in
27
28 248 bread volume by 1% - 2% β -glucan addition to dough ~~concluded the same results in GF~~
29
30 249 ~~doughs when water and β glucan varied concomitantly~~ (Lazaridou et al 2007).
31
32 250 Significant bread volume increases were also obtained when oat and barley dietary
33
34 251 fibres were added to GF doughs of adapted hydration (Sabanis et al. 2009). They also
35
36 252 ~~observed a maximum on bread specific volume with water, and obtained an additional~~
37
38 253 ~~increase in bread volume by 1% - 2% β glucan addition to dough.~~ Hydrocolloids can
39
40 254 improve dough development and gas retention by increasing dough viscosity (Houben,
41
42 255 Hoechstoeffer, and Becker 2012), but, there is an optimum value for the resistance to
43
44 256 deformation. Too high values can cause a limited and slow expansion of the gas cells
45
46 257 during proofing (Van Vliet et al 1992). ~~From the negative sign of the quadratic~~
47
48 258 ~~coefficients of BBG and SFE, a dose leading to a maximum in bread height and specific~~
49
50 259 ~~volume may be retrieved. For HPMC, besides water retention properties associated to~~
51
52 260 ~~hydrophilic nature, hydrophobic groups induce additional properties including increased~~
53
54 261 ~~interfacial activity within the dough system during proofing, and leading to gel network~~

~~formation on heating over the breadmaking process. Such network structures serve to increase viscosity and to further strengthen the boundaries of the expanding cells in the dough, thus increase gas retention through baking, and consequently lead to a better loaf volume (Bell 1990).~~

It is noteworthy that formulations yielding higher specific volume ~~and loaf height~~ in breads correspond to high doses of both SFE and water that gave poor quality breads with large gas cells (pictures not shown), in agreement with previous works (Haque and Morris 1994; McCarthy et al 2005; Nishita et al 1976) on high water GF breads. Authors related pocket formation to a poor dough consistency due to an excess of water.

~~Up to a certain level, a soft consistency, as promoted by high water addition and limited amounts of hydrocolloids, seems to be advantageous, allowing a larger increase in volume. However, The~~ excessive low consistency seems to cause the bubbles to become unstable, coalescing and resulting in large holes, after the crust has formed. ~~In preliminary tests, with individual additions of either SFE or NE, it was found that the occurrence of grain defect took place mainly for SFE breads and for water doses above 90%, flour basis.~~ Owing to the differences between the two types of HPMC used in the present work, defects could be related to the facility of crust forming that could be higher in the case of SFE (a semi-firm gel forming) than in NE (a weak gel forming) helping retain the large bubbles formed inside the crumb. With no exception, defects were only observed at water amounts above 90%.

Loss of weight

The loss of weight during baking varied between 26 g and 41 g, equivalent to 13% and 21% with respect to the initial amount of the baked dough ~~(Table 2)~~. Table 42 shows that the loss of weight depended mainly on the water content of the dough. The significant ($p < 0.05$) coefficients of the regression equation ~~(positive linear and negative~~

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

~~quadratic coefficients) obtained for water~~ indicate that water loss increased up to a
maximum with increased dough water content (Table 42). ~~When water content is low,~~
~~increasing water results in an increase of the rate of water lost by evaporation. However,~~
~~when the water content is high enough, above a critical amount an additional water~~
~~increase did not lead to an additional increase in the loss of weight, since the amount of~~
~~water lost during baking is determined by baking time that remained constant in this~~
~~study.~~ In absence of hydrocolloids the maximum loss of weight increase was 20% and
took place at a dough hydration of 94%. The marked effect of water content on loss of
weight during baking probably masked the effect of the remaining design factors.
~~Although no significant coefficients were obtained for SFE in the multifactor~~
~~regression, the comparison by pairs of the experimental results obtained for each run~~
~~(see runs 3, 7 and 9), allowed to confirm a significant increase ($p<0,05$) in the loss of~~
~~water during baking for increasing SFE amounts, provided remaining factors keep~~
~~constant (data not shown). Similar results were previously obtained in hydrocolloid-~~
~~added cake systems where HPMC was the only tested hydrocolloid that led to losses of~~
~~weight in baking above the control cake (without hydrocolloid) (Gómez et al 2007). The~~
individual addition of BBG to dough decreased the loss of weight during baking as
indicated by the negative linear significant coefficient. At a flour hydration level of
70%, the individual addition of 3.9 % BBG led to a 27% decrease in the loss of weight.
This was probably due to the high water binding capacity of β -glucans in line with
previous results (Brennan and Cleary 2007). However, the positive sign of the
BBG*WATER interaction coefficient anticipates that an increase in dough water
content decreased the individual ability of BBG to reduce the loss of weight during
baking. Accordingly, the amount of water that led to the maximum loss of weight in
BBG absence, 94%, increased ~~from 94%~~ to 104 % for 2% BBG and to 112% for 3.9%

BBG. The mentioned dough hydration values that maximized the loss of weight during baking also maximized the bread specific volume at each BBG addition.

Texture of the bread

Fresh bread crumb firmness varied from 1 N to 17 N (Table 2), which means a very wide range of crumb hardness among tested breads. From the multiple regression study, firmness was only significantly affected ($p < 0.05$) by both water and BBG (Table 4Table 2). These only two factors could explain 91% of the variability of fresh bread firmness, as indicates the R^2 value included in Table 4Table 2. The effect of cellulose derivatives, ~~which was detected by the analysis and comparison of individual experiences (Table 2),~~ was notably smaller than that associated to the other two ingredients. ~~The individual addition of SFE led to a decrease in firmness (runs 3, 7 and 9) that did not reach a significant level, while NE hardly showed any effect (runs 1, 5 and 10).~~ In absence of hydrocolloids, a significantly softer crumb was observed when the water content was increased from 70% to 90% ~~(Fig. 1e), as it was observed reported~~ earlier (McCarthy et al 2005). However, as can be predicted from the positive quadratic coefficient, additional amounts of water made crumb firmer again. Firmness evolution could be explained in parallel to specific volume evolution. In fact, both properties showed a significant ($p < 0.001$) negative correlation (see section 3.3). Water dosage that led to a minimum crumb firmness led to a concomitant maximum specific volume. The same could be reported from the BBG effect. Additional amounts of water in absence of hydrocolloids, probably decreased dough consistency too much and hindered dough gas retention ability during proofing and baking. The fact would explain the decrease in bread volume and the consequent increase in bread firmness.

The individual effect of BBG can be concluded from the high positive coefficient of the linear term of the regression equation (Table 4Table 2). ~~Accordingly, the individual~~

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

~~addition of BBG to the lowest hydration dough (70% flour basis, 0 level of water),~~
~~increased bread firmness more than seven times the initial firmness of bread (runs 2 and~~
~~8). Other authors observed that BBG increased firmness and decreased loaf volume of~~
~~wheat breads (Brennan and Cleary 2007; Cavallero et al 2002). These effects were~~
~~attributed to gluten dilution and to the BBG ability to binding appreciable amounts of~~
~~water, making it less available for the development of gluten network. Similar results~~
~~were obtained in GF bread related to the high water binding of BBG. However, the~~
significant negative interaction between water and BBG explains that the simultaneous
addition of BBG and water counteracted the individual effect of BBG. From the
predictive model, addition of 4% BBG with water content above 95 % would lead to
crumbs with a similar firmness to those obtained with doses of cellulose derivative
around 1.5 %. Fig. 1 shows the firmness of fresh breads and their evolution during nine
days of storage. It allows the comparison of runs where only varies a factor while the
rest are constant in the intermediate level of the range studied. The comparison of runs 7
and 11 shows the importance of dough hydration on BBG-enriched GF bread
development (~~Table 2~~Fig. 1c).
Chewiness of breads ranged 0.3 – 5.8 N and varied in parallel as hardness did (~~Table~~
~~4~~Table 2). This could be expected as both textural properties are directly related.
Cohesiveness (varied between 0.4 and 0.6) and springiness (ranged 0.6 – 0.9), also
involved in chewiness equation, showed minor effects. The resilience variation could
not be correlated to the studied factors, probably due to the low values and the low
variations observed between runs (0.19 – 0.29). Springiness~~or the after stress retarded~~
~~recovery capacity~~, increased with single addition of BBG and/or water (see significant
quadratic terms in Table 2).~~—~~ From the multivariate regression equation it could be
predicted a 21% increase in crumb springiness obtained from low hydration dough

(70% water) with 3% BBG and of 39% for 3.9% BBG. In absence of hydrocolloids the increase of dough hydration from 80% to 110% led to springiness increases around a 10% per each 10 % increase in dough water content. The negative interaction WATER*BBG (Table 4) explains that the simultaneous increase of water and BBG in dough did not lead to the expected springiness as result of the sum of the individual effect of Water and BBG.

Crumb and crust colour

Bread crust colour features showed bigger dependence on both hydrocolloid and water contents, than bread crumb. Bread crusts showed lightness values that ranged 60-74%. The a^* and b^* coordinates varied between 2–13 and 19–30 respectively (see Fig 2) leading to hue values that ranged of 64 – 84 degrees, and chromas between of 19 – 32 (Table 2 data not shown). Crust colours had higher L^* and b^* coordinates and lower a^* one than other authors found (Lazaridou et al, 2007) probably due to the use of corn as starch source in that work. This could be due to the presence of corn starch and caseinate in the formula of the previous work. In our case, the matrix was rice flour, of a very white colour, that hardly contributed to the bread colour. Attending the From multivariate regression equation (, from the positive quadratic term in Table 4 Table 2) it would be predictable a 10% increase in bread crust Lightness for the maximum dough hydration of 110% (40 level) with respect to the lowest one (70%). This was probably due to the reduction of Maillard reaction progress progress, main responsible for crust browning during baking, due as consequence of to this reaction precursor's dilution.

Single SFE incorporation addition to a dough with the minimum water content, 70%, to dough would lead to a 12% of maximum decrease in bread crust Lightness L^* at a dose of 2%. However, as it can be concluded from the negative regression coefficient. Decrease of available water in crust due to the SFE water binding in a matrix with

1
2
3
4
5
6 387 ~~limited amount of water (70% water, flour basis) may affect the Maillard reaction. An~~
7
8 388 additional dose of SFE did not showed additional effect ~~(Table 2), as predicts the~~
9
10 389 ~~positive coefficient of the quadratic term shown in Table 4. Probably, above 2%, SFE~~
11
12 390 ~~hardly hydrated, and did not contribute to Maillard reaction notably.~~ Single BBG
13
14 391 increased crust Lightness until 14% for the maximum dose tested, 3.9%, in accordance
15
16 392 with the positive quadratic coefficient in the regression equation. The effect, also
17
18 393 observed in previous works (Lazaridou et al 2007), could be explained by the already
19
20 394 mentioned BBG water retention capacity during baking, that led to breads with
21
22 395 significantly lower weight loss. Simultaneous increase of BBG and Water led to darker
23
24 396 bread crusts than expected for the individual action of BBG, ~~as indicated by the~~
25
26 397 ~~negative sign of the interaction~~ (significant BBG*Water coefficient), concomitant with
27
28 398 the increase in baking water loss already mentioned in the previous section.
29
30 399 Bread crust hues, ~~significantly –affected by all the factors studied (Table 2)~~
31
32 400 corresponded to more yellowish (h=90) than reddish (h=0) colours. ~~Bread crust hue was~~
33
34 401 ~~significantly affected by all the factors studied (Table 4).~~ It was observed that, in
35
36 402 general, breads with lower crust hues (more reddish) had lower Lightness values. The
37
38 403 ~~This is probably due to a common origin of both crust colour attributes. The~~
39
40 404 ~~circumstances that favoured Maillard reaction and decreased Lightness (SFE increase~~
41
42 405 ~~and the water/-BBG decrease)~~ led to reddish and darker bread crust. Lazaridou et al
43
44 406 (2007) also observed a tendency to yellowish in 1% oat β -glucan-added GF-bread crusts
45
46 407 (Lazaridou et al 2007). The crust hue was also significantly decreased by NE, although
47
48 408 in less extent than SFE, ~~as it can be concluded from the lower linear and quadratic~~
49
50 409 ~~regression coefficients (Table 4 Table 2).~~
51
52 410 Crumb colour, that mainly depends on the own ingredient colours, was not very affected
53
54 411 by the design factors due to the white-slight cream colour of added fibers. ~~Only the~~

chroma showed significant linear dependence on dough hydration and on viscous dietary fibres with a R^2 of 81.4%. ~~The a^* and b^* coordinates of all bread crumbs ranged from -0.23 to +0.44 and from +7 to +12 respectively. These values corresponded to crumb hues (h) from 87 to 91 degrees, which correspond to yellow colours. The chroma (C^*) values ranged 7 to 12. Dough hydration had the highest effect on crumb chroma.~~ With no added hydrocolloids the maximum water content increase (40%) led to 59% increase in the crumb ~~c~~chroma ~~(leading to the highest crumb chroma values (more vivid colours)s).~~ The presence of both types of HPMC counteracted the individual effect of water.

Bread staling

Bread staling was assessed by means of hardness evolution over 9 days (Figure ~~13~~). Experimental data were fitted to the Avrami equation (Armero and Collar 1998; Ronda and Roos 2011), and adjusted values were plotted leading to the continuous lines. Hardening during storage is expected as a result of moisture loss and starch retrogradation (Ronda et al 2011). Water was the factor with more prominent effect on GF bread staling, ~~as shown in Figure 31c. This figure shows that the bread made with the lowest water content, 70 % in flour basis reached, had after one storage day, a hardness ten times that of the bread with a 90% water content. After nine days of storage hardness was 80 N, which represents an extremely high value. It should be noted that the curves in Figure 3e correspond to constant concentrations of SFE, NE and BBG of 1.3%, 1.3% and 2 % respectively. the bread made with a water content of 70 % reached, after one storage day, a hardness ten times that of the bread with a 90% and the same hydrocolloid contents. After nine days of storage hardness was 84 N, which represents an extremely high value.~~ This demonstrates that hydrocolloids, with water restrictions, lead to unsuitable bread characteristics. The different effect of SFE and NE

on bread hardening was also remarkable. Figure 13b shows that SFE increase from 0.1% to 2.5% led to significant depletion in bread hardness during storage, in spite of 1.3% NE presence in bread formulation. On the contrary, same changes in the concentration of NE to a fixed concentration of 1.3% SFE hardly changed hardness curves (Figure 3b). SFE, therefore, showed a clearly better ability than NE to increase bread keepability. In previous studies it has been found that carboxymethylcellulose (CMC), added to GF doughs, controlled crumb hardening after three days storage (Lazaridou et al 2007).

As it can be seen in Fig. 1a, higher contents of BBG accelerated the aging of breads with 90% of water and 2.6% of HPMC (1.3% SFE + 1.3% NE). As it can be seen in Figure 3a, higher contents of BBG accelerated the aging of breads with 90% of water and 2.6% of HPMC (1.3% SFE + 1.3% NE). Other authors also observed this effect in breads added with β -glucan (Lazaridou et al 2007). Bread crumbs with 3.9% of BBG were since the second day of storage significant firmer than those with lower BBG addition (0.1%, 2%). This could be due to the This high dose of BBG would have required, attending its already commented higher water necessities of this high BBG dose (3.9%); a dough hydration near 110% as was shown before, instead of 90% to minimize crumb firmness. It is also noteworthy that the firming curves of breads with 2% and 3.9% of BBG did not reach a constant value in nine days, unlike the curve for breads with 0.1% BBG, which achieved in five days a stable value. 3.9% BBG delayed the achievement of the maximum hardness during storage at 4°C, generally attained in 6 – 8 days (Ronda and Roos 2011), probably due to the great water binding ability of BBG, controlling the loss of water during aging (Ronda et al 2011).

Table 2 shows the significant coefficients of the fitting model for the crumb hardening after one (short-term) and nine (long-term) days of storage at 4°C. The

regression study confirmed the individual effects of HPMC, BBG and Water on the already mentioned crumb hardening. ~~In short term storage (one day), SFE effect on crumb hardness reduction was much more markedly than NE. Attending the regression equation a dose of 2% SFE would lead to a 40% reduction in the increment of firmness after one storage day. Individual effect of BBG was the opposite: Doses of 2% and 3% led to an increase in the hardness after one day of 17% and 38% respectively. In long term storage, water content interactions with hydrocolloids exerted the most marked effects on crumb hardness. BBG was responsible for a significant crumb hardening after nine days of storage, but the provision of additional water content to the formula reduced significantly the effect.~~

3.2 Effect on Bread sensory quality

The coefficients of the regression fitting models for the sensory attributes ($0.76 \leq R^2 \leq 0.90$) are compiled in ~~Table 4~~ Table 2. ~~Panelists scored crumb grain for both cell size and distribution, giving low ratings to big and heterogeneously distributed alveoli and high ratings to small and homogeneous gas cells (Table 3).~~ SFE, BBG and ~~w~~water markedly affected crumb grain scores that varied between 2.4 ± 0.5 and 9.7 ± 0.5 in the scale 1-10. Negative coefficients of single BBG and/or Water (~~Table 4~~ Table 2) suggest both factors increased alveoli size and crumb grain heterogeneity, although BBG quadratic coefficient supports that from 1.7 % BBG addition crumb grain ratings became higher. SFE and Water showed a significant negative interaction on crumb grain scores, so that, high SFE dosages to softer doughs led to a prominent decrease in crumb grain ratings (~~Table 3~~). Lower crumb grain scores were concomitant with the presence of big holes (pockets) in bread crumb, and unaccepted by panelists. Ratings for crumb softness ~~and smoothness (Table 3)~~ were ~~parallel and~~ dependent on Water, BBG and SFE (Table 24). Increased water amounts up to 95 -100% hydration in dough

Formatted: Font: Bold, Not Italic

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

promoted significantly sensory ratings for crumb ~~smoothness~~softness. Higher hydration levels did not provide any additional ~~sensory smoothness~~ perception. BBG increasing single addition decreased ~~smoothness~~softness regardless dough water content. Simultaneous SFE presence helped BBG increase crumb ~~smoothness~~softness when added up to 2%, flour basis. In fact, SFE counteracted BBG deleterious effect on crumb smoothness, so that SFE appears as a key ingredient in BBG-enriched GF-breads.

Overall acceptance of GF-breads, that varied between 2.2 $\pm$ 0.4, for Run 13, and 7.6 $\pm$ 0.4, for Run 12, was only significantly affected ($p < 0.05$) by water and BBG dosages. According to panelits' scores, overall acceptance was mainly/significantly dependent on crumb grain, ~~and~~ softness~~and smoothness~~ (Table 6Table 4). Breads with water formulation greater/equal than 90% (flour basis) deserved acceptance ($\geq 5/9$), with the exception of Run 13, including 3.9 % BBG. It should be noticed that the bread of Run 12 (3.34% BBG, 0.45% SFE, 2.15% NE, 104% WATER), was individually preferred (7.6 $\pm$ 0.4/9). The ratio BBG/Water of this formulation coincided with that predicted from the regression equations as a ratio capable of optimizing the bread quality leading to minimum bread firmness and maximum specific volume.

3.23 Correlation between variables.

Multivariate data handling of dough visco-elastic and bread quality parameters supplied useful information on the significantly correlated dough and bread characteristics. Fundamental and empirical dough rheological properties were reported in a previous work (Ronda et al 2013). Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.46 to 0.90) was obtained for the relationships between dough viscoelastic parameters and bread properties of fibre-supplemented rice-based matrices (Table 5Table 3). Table 6Table 4 reports Pearson correlation coefficients for the relationships between bread properties. Bread specific volume strongly correlated with

dough viscosity at the steady state, η_0 , obtained from creep tests ($p < 0.001$, $r = -0.76$). Specific volume explicated a negative correlation with the elastic modulus at 1 Hz, G_1' , ($r = -0.64$) and positive with loss tangent, $\tan \delta$, and the exponents "a" and "b" ($r = 0.56$, $r = 0.53$, $r = 0.56$). The exponents, resulting from fitting power law to oscillatory frequency sweep data, quantify the dependence of dough elastic and viscous moduli on frequency. The higher the dependence of dynamic moduli on the frequency is, the higher $\tan \delta$ and the greater the bread volume. This means that lower dough consistency and less marked solid-like behaviour (within the range $0.19 < \tan \delta < 0.48$) favoured dough development and bread volume. Baking weight loss was strongly correlated with dough visco-elastic moduli, G_1' and G_1'' , ($p < 0.001$; $r = -0.86$; $r = -0.78$), the viscosity at the steady state, η_0 , ($p < 0.01$; $r = -0.68$) and the dough consistency or the force obtained in a back extrusion test ($p < 0.001$; $r = -0.82$). The greater the dough consistency means the higher the baking yields. This is probably due to the fact that fibres, substances responsible for the dough consistency increase, were also good water-binding macromolecules. Fresh bread crumb firmness and one- and nine-days stored bread crumb hardening were strongly correlated to all visco-elastic dough properties. The strongest positive correlation was obtained for fresh bread crumb firmness and the elastic modulus, G_1' ($p < 0.001$; $r = 0.79$), and the viscosity at steady state, η_0 , ($p < 0.001$; $r = 0.88$). The short- and long-term bread hardening exhibited similar relationships with dough properties than initial firmness. Sensory scores related to crumb grain, crumb softness and overall acceptance were strongly correlated ($p < 0.001$) with fundamental and empirical dough rheological properties.

4. Conclusions

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

535 Draper-Lin small composite design for sampling allow to obtain useful information on
536 the significance of different hydrocolloid and water contents on breadmaking
537 performance and staling behaviour of GF rice-based complex/heterogeneous bread
538 matrices. Dough and bread characteristics strongly correlated supporting the key
539 influence of the restoration of dough visco-elasticity on the physico-chemical and
540 sensory achievement of GF final baked goods.

541 Single BBG by itself fails to mimic gluten visco-elasticity properly, but simultaneous
542 addition of either SFE or NE, in doses around 1.6 %, contribute to bread improvement
543 in terms of bigger volume and smoother crumb. ~~The extent of methoxyl and~~
544 ~~hydroxypropyl substitution of HPMC markedly affects the gel strength.~~ Weak gel
545 forming NE led to harder and lower volume breads than semi-firm gel forming SFE did,
546 probably ascribed to the ability of hydroxypropyl groups to form a stable solvate shell
547 in water restricting available water for starch to swell. It should be noticed that the
548 surplus of water needed to incorporate large amounts of BBG into bread favoured the
549 formation of big holes (pockets) in bread crumb particularly when SFE ~~is~~was included
550 into formulation. Visible holes could be probably related to the easier crust forming
551 ability of SFE than NE helping retain the large bubbles formed inside the crumb. A
552 dough hydration of 90% would be recommended to get the maximum volume of no
553 BBG added breads. This optimal amount of water would increase at a rate of 5-6% per
554 1% increase in BBG.

555 3.3 g of ~~BBG~~BBG (70% purity) and 104mL of water addition to 100 g rice flour
556 provided sensorially accepted breads ($7.6 \pm 0.4/9$) over 10 with a ~~theoretical~~ theoretical β -glucan
557 content of 1.24 g/100 g GF bread. It would allow a daily intake of 3 β -glucan
558 provided with a bread consumption of 240 g/day. ~~A daily intake of 240 g of BBG~~
559 ~~enriched GF bread (four servings), in good accordance with the nutritional~~

~~recommendation for bread consumption set between 200 and 300 g of bread per day (Zessner et al 2011), is high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a reduced blood cholesterol level.~~

~~An additional refined optimization of both water and quali and quantitative HPMC addition for BBG concentrations from 3 % to 4 %, is still recommended~~Complementary tests aimed at establishing the real content and molecular weight of - β -glucan in final bread are still pending to assure the expected nutritional benefit of thisBBG enrichment to obtain healthy GF breads with improved organoleptic performance.

Acknowledgement

Authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad (Projects AGL2012-35088 and AGL2011-22669) and Comunidad de Castilla y León (Project VA252A12-2), the laboratory of Pharmacy (laboratory accredited according to ISO 17025) of the University of Basque Country for the gluten analysis in barley β -Glucan extracts, ~~and Cargill Company for the delivery of Barley Beta-glucan.~~

References

- Anton, A. A. (2008). Improving the nutritional and textural properties of wheat flour tortillas. *Cereal Research Communications*, 36, 301-311.
- Armero, E. & Collar, C. (1998). Crumb firming kinetics of wheat breads with anti-staling additives. *Journal of Cereal Science*, 28, 165-174.
- Bell, D. A. (1990). Methylcellulose as a ~~S~~structure ~~E~~enhancer in ~~Bread-bread Bakingbaking~~. *Cereal Foods World*, 35, 1001-&.
- Brennan, C. S. & Cleary, L. J. (2007). Utilisation of Glucagel-~~(R)~~ in the beta-glucan enrichment of breads: A physicochemical and nutritional evaluation. *Food Research International*, 40, 291-296.

Formatted: Space Before: 12 pt, After: 0 pt

Formatted: Font: Bold

1
2
3
4
5
6 585 Cavallero, A., Empilli, S., Brighenti, F. & Stanca, A. (2002). High (1 → 3,1 → 4)-beta-
7 586 glucan barley fractions in bread making and their effects on human glycemic response.
8
9 587 *Journal of cereal science*, 36, 59-66.

10
11 588 Collar, C., Andreu, P., Martinez, J. & Armero, E. (1999). Optimization of hydrocolloid
12
13 589 addition to improve wheat bread dough functionality: a response surface methodology
14
15 590 study. *Food Hydrocolloids*, 13, 467-475.

16
17 591 Collar, C., Santos, E. & Rosell, C. M. (2005). Providing healthier baked goods through
18
19 592 high fibre optimised formulations: Functional requirements. In: *Intradfood Innovations*
20 593 *in Traditional Foods* (edited by P. Fito & F. Toldrá). Pp. 879-882. London: Elsevier.

21
22 594 Collar, C., Bollain, C. & Angioloni, A. (2005). Significance of microbial
23
24 595 transglutaminase on the sensory, mechanical and crumb grain pattern of enzyme
25 596 supplemented fresh pan breads. *Journal of Food Engineering*, 70, 479-488.

26
27 597 Collar, C., Santos, E. & Rosell, C. M. (2007). Assessment of the rheological profile of
28
29 598 fibre-enriched bread doughs by response surface methodology. *Journal of Food*
30 599 *Engineering*, 78, 820-826.

31
32 600 Draper, N. R. & Lin, D. K. J. (1990). Small ~~Response~~response-Surface-surface
33 601 ~~Designs~~designs. *Technometrics*, 32, 187-194.

34
35
36 602 EFSA. (2011). Scientific Opinion on the substantiation of health claims related to beta-
37
38 603 glucans from oats and barley and maintenance of normal blood LDL-cholesterol
39 604 concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy
40 605 intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and
41 606 “digestive function” (ID 850) pursuant to Article 13(1) of Regulation (EC) No
42 607 1924/2006. *EFSA Journal*, 9, 2207.

43
44
45 608 Gill, S., Vasanthan, T., Ooraikul, B. & Rossnagel, B. (2002). Wheat bread quality as
46
47 609 influenced by the substitution of waxy and regular barley flours in their native and
48
49 610 extruded forms. *Journal of cereal science*, 36, 219-237.

50
51 611 ~~Gomez, M., Ronda, F., Caballero, P. A., Blanco, C. A. & Rosell, C. M. (2007).~~
52 612 ~~Functionality of different hydrocolloids on the quality and shelf life of yellow layer~~
53 613 ~~cakes. Food Hydrocolloids, 21, 167-173.~~

- Haque, A. & Morris, E. R. (1994). Combined use of ~~h~~spaghula and ~~HPMC~~me to ~~R~~replace ~~O~~r ~~a~~Augment ~~G~~gluten in ~~B~~breadmaking. *Food Research International*, 27, 379-393.
- Haque, A., Richardson, R. K., Morris, E. R. & Dea, I. C. M. (1993). Xanthan-~~Like-like~~ ~~Weak-weak~~ ~~Gel-gel~~ ~~Rheology-rheology~~ from ~~Dispersions-dispersions~~ of ~~Ispaghula~~ ~~ispaghula~~ ~~Seed-seed~~ ~~Huskhusk~~. *Carbohydrate Polymers*, 22, 223-232.
- Houben, A., Hoechstotter, A. & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology*, 235, 195-208.
- Kinner, M., Nitschko, S., Sommeregger, J., Petrasch, A., Linsberger-Martin, G., Grausgruber, H., Berghofer, E. & Siebenhandl-Ehn, S. (2011). Naked barley-~~O~~ptimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. *Journal of cereal science*, 53, 225-230.
- Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N. & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*, 79, 1033-1047.
- McCarthy, D., Gallagher, E., Gormley, T., Schober, T. & Arendt, E. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry*, 82, 609-615.
- Nishita, K. D., Roberts, R. L., Bean, M. M. & Kennedy, B. M. (1976). Development of a ~~Yeast~~cast-~~Leavened-leavened~~ ~~Rice~~rice-~~Bread-bread~~ ~~Formula~~formula. *Cereal Chemistry*, 53, 626-635.
- Ronda, F., Perez-Quirce, S., Angioloni, A. & Collar, C. (2013). Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological approach. *Food Hydrocolloids*, 32, 252-262.
- Ronda, F. & Roos, Y. H. (2011). Staling of fresh and frozen gluten-free bread. *Journal of cereal science*, 53, 340-346.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

641 Ronda, F., Gomez, M., Blanco, C. A. & Caballero, P. A. (2005). Effects of polyols and
642 nondigestible oligosaccharides on the quality of sugar-free sponge cakes. *Food*
643 *Chemistry*, 90, 549-555.

644 Ronda, F., Caballero, P. A., Quilez, J. & Roos, Y. H. (2011). Staling of frozen partly
645 and fully baked breads. Study of the combined effect of amylopectin recrystallization
646 and water content on bread firmness. *Journal of cereal science*, 53, 97-103.

647 Sabanis, D., Lebesi, D., Tzia, C. (2009). Effect of dietary fibre enrichment on selected
648 properties of gluten-free bread. *Food Science and Technology*, 42, 1380-1389.

649 Thomson, T. (2009). The nutritional quality of gluten-free foods. In: *Gluten-Free Food*
650 *Science and Technology* (edited by E. Gallagher). Pp. 45-51. New Delhi: Wiley-
651 Blackwell.

652 Van Vliet, T., Janssen, A. M., Bloksma, A. H. & Walstra, P. (1992). Strain-~~Hardening~~
653 ~~hardening~~ of ~~Dough—dough~~ as a ~~Requirement—requirement~~ for ~~Gas—gas~~
654 ~~Retentionretention~~. *Journal of Texture Studies*, 23, 439-460.

655 Wood, P. J. (2010). Oat and Rye beta-~~Glucan~~glucan: Properties and ~~Functionfunction~~.
656 *Cereal Chemistry*, 87, 315-330.

657 Zessner, M., Helmich, K., Thaler, S., Weigl, M., Wagner, K. H., Haider, T., Mayer, M.
658 M. & Heigl, S. (2011). Ernährung und ~~Flächennutzung—flächennutzung~~ in Österreich.
659 *Öwaw*, 5/6, 95-104.

Figure 13: Crumb firmness evolution during 9 days of storage time at 4°C of breads with different amounts of BBG (runs 5, 7 and 13) (a) HPMC (runs 1, 3, 7, 9 and 10) (b) and Water (runs 7, 11 and 14) (c). Intermediate doses of dietary fibres (1.3% SFE; 1.3% NE; 2% BBG) and water (90% WATER) were used as constant factors. Values of firmness are the average of four replicates and error bars represent standard deviation.

Formatted: Font: Not Bold

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

For Peer Review

Table 1: Draper-Lin small composite design for sampling

Run	SFE	NE	BBG	WATER
1	0	-1.4142	0	0
2	-1	-1	-1	-1
3	-1.4142	0	0	0
4	1	-1	1	1
5	0	0	-1.4142	0
6	1	1	1	-1
7	0	0	0	0
8	-1	-1	1	-1
9	1.4142	0	0	0
10	0	1.4142	0	0
11	0	0	0	-1.4142
12	-1	1	1	1
13	0	0	1.4142	0
14	0	0	0	1.4142
15	0	0	0	0
16	1	-1	-1	1
17	-1	1	-1	1
18	1	1	-1	-1
19	0	0	0	0

Design factors are: hydroxypropylmethylcellulose semi-firm gel forming HPMC SFE 4000 (SFE), hydroxypropylmethylcellulose weak gel forming HPMC NE 4000 (NE), Barley Beta-Glucan (BBG), and water addition (WATER). -1.4142, -1, 0, 1, and 1.4142 indicate coded levels of design factors; axial distance from the center points: 1, 1.4142.

Table 2: Significant coefficients (95% confidence interval) of design factors (independent variables) of the stepwise regression fitting model for bread characteristics (dependent analytical variables).

Factor	Specific volume (ml/g)	Height/ width	Loss of weight (g)	Firmness (N)	Sprin- giness	Chewiness (N)	Crust L*	Crust h	Crust C*	Crumb C*	ΔFirmness 1 day (N)	ΔFirmness 9days (N)	Crum grain (Score)	Crumb softness (Score)	Overall Acceptance (Score)
CONSTANT	1.41	0.30	31.1	2.52	0.71	0.71	71.6	86.4	86.4	11.7	20.7	10.7	10.8	1.8	2.3
SFE	1.54	0.29			-0.054		-8.69	-16.0	-16.0		-5.6				
NE								-9.2	-9.2						
BBG	0.14		-2.1	4.98		1.97						74.0	-1.8		
WATER	0.10	0.017	0.51	-0.50		-0.18					-1.1	-6.5	-0.10	0.35	0.29
SFE ²	-0.48	-0.096					2.22	2.9	2.9						
NE ²								1.7	1.7						
BBG ²	-0.24	-0.025			0.017		0.67	0.82	0.82		0.87		0.52	-0.21	-0.12
WATER ²	-0.0026	-0.0006	-0.011	0.015	0.00018	0.0057	0.0070	0.0046	0.0046	0.0043	0.020	0.10		-0.0045	-0.0038
SFE*NE	0.15							3.3	3.3						
SFE*BBG		-0.031										-13.1		0.24	
SFE*WATER		0.0056								-0.079	0.16	1.2	-0.045		
NE*BBG		0.028					-0.71	-1.4	-1.4	0.23		-14.3			
NE*WATER										-0.086		1.3			
BBG*WATER	0.029	0.0028	0.10	-0.17	-0.0031	-0.068	-0.11	-0.058	-0.058		-0.13	-1.3			
R-SQ	0.91	0.99	0.77	0.91	0.70	0.92	0.75	0.95	0.95	0.81	0.94	0.95	0.88	0.91	0.76

Independent variables: SFE= HPMC SFE 4000; NE= HPMC NE 4000; BBG: Barley Beta-Glucan; WATER= water content above 70%. Blanks correspond to no significant effects at level of 5%; R-SQ adjusted square coefficient of the fitting model

Table 3: Pearson product moment correlations between pair of variables measured in dough and bread. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. The rheological properties correspond to the fitting of experimental oscillatory measurements to power law model ($G' = G'_1 \cdot \omega^a$; $G'' = G''_1 \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \omega^c$). Creep test results to the 6-parameter Burgers model (where J_0 and μ_0 are the instantaneous compliance and the steady state viscosity respectively); Consistency is the firmness measured in an extrusion empirical test (Ronda et al. 2013).

	Specific volume	Loss of weight	Firmness	Δ Firmness 1 day	Δ Firmness 9days	Cohesiveness	Chewiness	Crumb grain	Crumb softness	Overall Acceptability
G'₁	-0.64**	-0.86***	0.79***	0.69***	0.59**	ns	0.79***	0.70***	-0.83***	-0.79***
a	0.53*	ns	-0.47*	-0.58**	-0.51*	0.49*	ns	-0.57**	0.65**	0.59**
G''₁	ns	-0.78***	0.61**	0.49*	ns	ns	0.63**	0.61**	-0.72***	-0.69**
b	0.56*	0.72***	-0.67**	-0.65**	-0.54*	0.54*	-0.65**	-0.74***	0.88***	0.81***
$\tan \delta_1$	0.56*	ns	-0.48*	-0.58**	-0.50*	0.54*	ns	-0.62**	0.68**	0.60**
c	ns	0.61**	ns	ns	ns	ns	ns	ns	0.53*	0.49*
J₀-creep	ns	ns	ns	ns	ns	0.62**	ns	-0.66**	0.62**	0.50*
μ_0-creep	-0.76***	-0.68**	0.88***	0.85***	0.90***	-0.49*	0.84***	0.70***	-0.83***	-0.74***
Consistency	-0.50*	-0.82***	0.58**	0.56*	ns	ns	0.61**	0.67**	-0.77***	-0.75***
Peak Temperature	0.57*	ns	-0.50*	-0.49*	-0.46*	ns	-0.52*	ns	ns	ns

Asterisks indicate the P-value which tests the statistical significance of the estimated correlations. *p<0.05 (statistically significant non-zero correlations at the 95.0% confidence level); ** p<0.01 (at the 99.0% confidence level); *** p<0.001 (at the 99.9% confidence level). ns: Not significant (p > 0.05)

Table 4: Pearson product moment correlations between pair of variables measured in bread. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables.

	Loss of weight	Firmness	ΔFirmness 1 day	ΔFirmness 9days	Chewiness	Crust Lightness	Crust hue	Crust Chroma	Crumb grain	Crumb softness	Overall Acceptance
Specific volume	0.83***	-0.80***	-0.83***	-0.72***	-0.80***	-0.49*	-0.56*	0.62**	-0.72***	0.73***	0.73***
Loss of weight		-0.75***	-0.74***	-0.55*	-0.77***	ns	ns	ns	-0.75***	0.76***	0.83***
Firmness			0.92***	0.91***	0.99***	ns	ns	-0.61**	0.55*	-0.73***	-0.62**
ΔFirmness 1 day				0.87***	0.90***	ns	0.53*	-0.65**	0.64**	-0.81***	-0.71***
ΔFirmness 9 days					0.85***	ns	0.65**	-0.61***	0.46*	-0.64**	-0.51*
Chewiness						ns	ns	-0.57*	0.55*	-0.71***	-0.62**
Crust Lightness							0.72***	-0.56*	ns	ns	ns
Crust hue								-0.78***	ns	ns	ns
Crust Chroma									ns	ns	ns
Crumb grain										-0.83***	-0.83***
Crumb softness											0.91***

Asterisks indicate the P-value which tests the statistical significance of the estimated correlations. *p<0.05 (statistically significant non-zero correlations at the 95.0% confidence level); ** p<0.01 (at the 99.0% confidence level); *** p<0.001 (at the 99.9% confidence level). ns: Not significant (p > 0.05)