**Lipid dynamics in blended wheat and non-wheat flours
breadmaking matrices: impact on fresh and aged composite
breads.**

Journal:	<i>Food Science and Technology International</i>
Manuscript ID	FSTI-16-0288.R1
Manuscript Type:	Research Article
Subject category:	Physical, Chemical and Sensory Properties
Date Submitted by the Author:	n/a
Complete List of Authors:	Collar, Concha; CSIC, Instituto de Agroquímica y Tecnología de Alimentos Conte, Paola
Keywords:	Breadmaking, Quality assessment, Lipids
Abstract:	<p>The use of pseudocereals, legumes and ancient grains for breadmaking applications is receiving particular attention since they involve nutrient dense grains with proven health-promoting attributes. Dilution up to 45% of the basic wheat flour matrix by accumulative ternary addition of teff, green pea and buckwheat flours did significantly impact both the extractability and distribution of lipid subfractions in composite flours, doughs and breads, and induced differentiated dynamics in lipid binding along breadmaking. During mixing, a preferential covalent lipid binding to the inside part of the starch granules takes place at the expenses of both accessible free lipids and lipids initially bound non-covalently to the gluten/non gluten proteins and to the outside part of the starch granules. During fermentation and later baking a preferential lipid binding to the gluten/non gluten proteins and to the outside and inside starch granules takes place at the expenses of both a free lipid displacement and a bound lipid translocation to new protein and starch active sites. It can be noticed that the larger the accumulation of both protein- and starch-bound lipids over fermentation and baking, the higher physic-chemical and sensory profiles, and the slower starch hydrolysis, firming and retrogradation kinetics of composite breads were obtained.</p>

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

SCHOLARONE™
Manuscripts

For Peer Review

Lipid dynamics in blended wheat and non-wheat flours breadmaking matrices: impact on fresh and aged composite breads.

Concha Collar^{1*}, Paola Conte²

¹Cereals and Cereal-based Products, Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos (CSIC). Avda. Catedrático Agustín Escardino, 7. 46980 Paterna. SPAIN.

²Dipartimento di Agraria. Sezione di Scienze e Tecnologie Ambientali e Alimentari. Università degli Studi di Sassari. Viale Italia, 39. 07100 Sassari. ITALY

*Corresponding author. Tel.: +34 963 90 00 22; Fax: +34 963 63 63 01

E-mail address: ccollar@iata.csic.es

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

Abstract The use of pseudocereals, legumes and ancient grains for breadmaking applications is receiving particular attention since they involve nutrient dense grains with proven health-promoting attributes. Dilution up to 45% of the basic wheat flour matrix by accumulative ternary addition of teff, green pea and buckwheat flours did significantly impact both the extractability and distribution of lipid subfractions in composite flours, doughs and breads, and induced differentiated dynamics in lipid binding along breadmaking. During mixing, a preferential covalent lipid binding to the inside part of the starch granules takes place at the expenses of both accessible free lipids and lipids initially bound non-covalently to the gluten/non gluten proteins and to the outside part of the starch granules. During fermentation and later baking a preferential lipid binding to the gluten/non gluten proteins and to the outside and inside starch granules takes place at the expenses of both a free lipid displacement and a bound lipid translocation to new protein and starch active sites. It can be noticed that the larger the accumulation of both protein- and starch-bound lipids over fermentation and baking, the higher physic-chemical and sensory profiles, and the slower starch hydrolysis, firming and retrogradation kinetics of composite breads were obtained.

Keywords. Composite Bread . Nutritional quality · Functional properties . Lipid dynamics

1. Introduction

Lipids provide a wide array of beneficial properties during breadmaking stages and bread storage which reflects the overall lipid diversity. Over the past years, the role of lipids in the areas of gas cell stabilisation (Sroan and MacRitchie, 2009) and the emulsifying properties of specific polar lipids (Selmair and Koehler, 2010) has been particularly emphasized. Lipids have a significant effect on the quality and texture of baked goods because of their ability to associate with proteins, due to their amphipathic nature (hydrophilic and hydrophobic groups present), and with starch, forming inclusion complexes (Goesaert et al., 2005). Nonpolar lipids are either associated with the glutenin polymeric network through hydrophobic interactions or entrapped within the gluten matrix. In gluten, glycolipids are likely to be associated with glutenins through both hydrophobic interactions and hydrogen bonds whilst phospholipids preferentially interact with gliadins and lipid binding proteins (McCann et al., 2009). Amylose-lipid complexation can prevent the cocrystallization of amylose with amylopectin, and lipids can also complex with the outer branches of amylopectin and inhibit retrogradation in a more direct way (Putseys et al., 2010).

In breadmaking applications, protein and starch lipid binding in both wheat flour and additive-bread systems have been reported to correlate with loaf volume, crumb structure, softness and/or texture of bread (Collar et al., 2001; Angioloni & Collar, 2011a). At dough level and in presence of surfactants, free and bound lipids preferentially bind to gluten (monoglycerides) and to the outside part of the starch granules (cationic surfactants). Hydrocolloids preferentially bind to the gluten and to the outside part of the starch granules depending on their polarity (Collar et al., 1998). In wheat bread, a preferential binding of the added anionic surfactant to the starch with a concomitant displacement of endogenous polar lipids from starch to gluten was observed (Collar et al., 2001).

The binding of free lipids with gluten proteins may provide lipids with the ability to align at the interface of gas cells during the initial phases of dough mixing and increase gas cell stability throughout the bread

1
2
3 72 making process (Pareyt et al., 2011). Gas cells are retained in dough during fermentation and baking. Both
4
5 73 gluten and lipids play a role in this. Initially, during fermentation, the growing gas cells are surrounded
6
7
8 74 (Selmair & Koehler, 2010) and stabilised by a continuous gluten network, the viscosity of the dough liquor
9
10 75 and the adsorbed lipid crystals. The gluten matrix remains only as a thin film between gas cells at the end of
11
12 76 fermentation and some surface active proteins and lipids exert film stabilising properties and, as such,
13
14 77 increase gas cell retention (Gan et al., 1995). The endogenous lipids and gluten proteins act synergistically
15
16 78 to stabilise the gas cells during fermentation.
17

18
19
20 79 In diluted wheat flour systems by addition of non-gluten forming grain flours, lipid binding during
21
22 80 breadmaking stages takes place to both gluten and non-gluten proteins and to mixed starches. In single and
23
24 81 blended oat, rye, buckwheat and wheat flour matrices, lipids bound to proteins during dough mixing are
25
26 82 translocated and bound to starch during baking. Lipids bound to starch showed the most significant
27
28 83 correlations with parameters related to dough and bread performance during breadmaking, especially over
29
30 84 the mixing step (Angioloni & Collar, 2011a). In complex grain flour matrices, during baking, lipids bind to the
31
32 85 gluten/non gluten proteins at the expenses of both a free lipid displacement and a lipid migration from the
33
34 86 inside part of the starch granules to the protein active sites. It was observed that the higher the decrease of
35
36 87 free lipid content during baking, the higher the pasting temperature and the lower the total setback on
37
38 88 cooling and the dynamic moduli, but the higher the specific volume in blended breads (Collar and Angioloni,
39
40 89 2014).
41
42
43
44

45 90 Pseudocereals and teff flours exhibit richer qualitative and quantitative lipid profiles than green pea and
46
47 91 wheat flours (Hager et al., 2012). This research is aimed at characterising the lipid fractions of flour, dough
48
49 92 and bread stages in single and blended teff, green pea and buckwheat added to a wheat flour matrix. An
50
51 93 analysis of the impact of free lipids and starch- and protein-lipid binding along the breadmaking steps on the
52
53 94 physico-chemical and sensory properties and starch digestibility of composite matrices was presented.
54
55
56
57
58
59
60

2. Experimental

2.1. Materials

Commercial flours from refined common wheat *Triticum aestivum* (W), and whole teff *Eragrostis tef* (T), green pea *Pisum sativum* (GP), and buckwheat *Fagopyrum esculentum* (BW) were purchased from the Spanish market. Protein, dietary fibre and fat contents (% flour, dry basis) were 14.13%, 2.19%, 1.56 (W); 25.12%, 14.56%, 1.27 (GP); 19.71%, 13.52%, 3.44% (BW), and 13.05%, 12.19%, 5.06% (T), respectively (Collar et al., 2014a). Refined W flour (70% extraction rate) of 356×10^{-4} J energy of deformation W, 0.64 curve configuration ratio P/L, 95% Gluten Index, 62% water absorption in Brabender Farinograph, was used. Ireks Vollsauer sour dough was from Ireks (Spain); Novamyl 10000 a maltogenic thermostable α -amylase of 10,000 Maltogenase Units (MANU) of activity, from Novozymes (Denmark); and calcium propionate, from Sigma-Aldrich (USA).

2.2. Methods

Bread making of wheat and wheat-based blended flours

Doughs and breads were prepared from W as control, and wheat-based blended flours (T, GP, BW) by W flour replacement from 22.5% up to 45%, and incorporation of ternary blends of T, GP and BW flours according to a Multilevel Factorial Design (Statgraphics Centurion XV, version 15.2.11, Statpoint Technologies, Inc. Warrenton, Virginia, USA) with the following attributes: 3 experimental factors (T, GP and BW flours) at 2 levels, coded 0 (7.5% W flour replacement) and 1 (15% W flour replacement), and 5 error degrees of freedom. The model resulted in 8 randomized runs in 1 block. A 3 digit bread sample code was set referring to low (0) and high (1) W flour replacement by T (1st digit), GP (2nd digit), and BW (3rd digit) flours in sample formulation, as it follows: 010, 001, 011, 000, 111, 101, 100, 110. Blended flours, water, commercial compressed yeast, salt, sugar, commercial sour dough, milk powder, Novamyl 10000, and calcium propionate were mixed, and used to make control and blended breads according to the quantitative

1
2
3 120 formulations and breadmaking procedure described earlier (Collar et al., 2014a). Bread samples were
4
5 121 stored for 1, 3, 6, and 8 days to describe firming kinetics. Two trials of baking tests per sample were
6
7
8 122 performed.

9
10 123
11
12
13 124 *Sensory analysis*

14
15 125 Sensory analysis of fresh breads was performed with a panel of ten trained judges (five males and
16
17 126 five females aged 24–57) using semi structured scales, scored 1–10 in which extremes (lowest:1; highest:
18
19 127 10) were described for each sensory attribute according to Setser (1996). Evaluated attributes were
20
21 128 grouped into visual (cell uniformity, size, brightness and shape, and cell wall thickness), textural (tactile
22
23 129 moistness, elasticity and smoothness, and biting coarseness, adhesiveness, cohesiveness, chewiness,
24
25 130 crumbliness and dryness) and organoleptic (taste intensity, quality, saltiness, sourness and aftertaste, and
26
27 131 aroma intensity, quality and sourness) characteristics. (Collar et al., 2005).

28
29
30 132
31
32
33 133 *Starch digestibility*

34
35 134 *In vitro* starch hydrolysis kinetics and relevant starch fractions in freeze-dried and ground fresh
36
37 135 blended breads was determined following the AACC (2005) method 32-40, adapted by Angioloni & Collar
38
39 136 (2011b) as previously described (Collar et al., 2014a). Rapidly Digestible Starch (RDS) and Slowly
40
41 137 Digestible Starch (SDS) were measured after incubation for 20 min and 120 min, respectively. Digestible
42
43 138 starch (DS) was calculated by the sum of RDS and SDS. Total starch (TS) was calculated by the sum of DS
44
45 139 and RS. A first order kinetic equation $[C = C_{\infty} (1 - e^{-kt})]$ was applied to describe the kinetics of starch
46
47 140 hydrolysis, where C , C_{∞} and k were the hydrolysis degree at each time, the maximum hydrolysis extent and
48
49 141 the kinetic constant, respectively. Resistant starch (RS) determination was performed according to AOAC
50
51 142 Official Method 2002.02 (AOAC, 2000) and AACC Method 32-40 (AA CC, 2005), as described by
52
53
54
55
56
57
58
59
60

143 Megazyme International Ireland (2011) in the Resistant Starch Assay Procedure (kit K-RSTAR 08/11).

144 Three replicates were made per analysis.

145

146 *Textural measurements and firming kinetics*

147 Bread mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads
148 were recorded in a TA-XTplus texture analyser (Stable Micro Systems, Surrey, UK) using a 25 mm diameter
149 cylindrical aluminium probe, a 5 kg load cell, 50% penetration depth at a running speed of 1mm/s, and a 30
150 s gap between compressions on crust-free slices of 25 mm width (Armero & Collar, 1998). For textural
151 measurements, three slices of two breads were used for each sample. The obtained firming curves during
152 bread storage were modelled using the Avrami equation, and model factors were estimated by fitting
153 experimental data of hardness to the nonlinear regression equation $\theta = \frac{T_{\infty} - T_t}{T_{\infty} - T_0} = e^{-kt^n}$ where θ is the
154 fraction of the recrystallisation still to occur; T_0 , T_{∞} and T_t are crumb firmness at time zero, ∞ and time t ,
155 respectively, k is a rate constant, and n is the Avrami exponent.

156

157 *Thermal measurements and retrogradation kinetics*

158 Thermal properties regarding starch retrogradation of control and blended samples were assessed
159 in a Differential Scanning Calorimeter Perkin-Elmer DSC-7 (Norwalk, USA) as previously reported by Collar
160 et al. (2015). Stored previously gelatinized dough samples (Collar et al., 2015) were submitted to a second
161 DSC scan to analyze starch retrogradation at the different storage periods after 1, 3, 6, and 8 days.
162 Scanning conditions included keeping sample pans at 25°C for 1 min, and then heating from 25 to 130°C at
163 a rate of 10°C/min. The enthalpy of amylopectin retrogradation (ΔH_r) was calculated, and T_0 , T_p , and T_e for
164 the thermal transitions, identified. All samples were analyzed in duplicate. Modelling of crystallization data
165 was carried out using the Avrami equation, and model factors were estimated by fitting experimental data

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

for melting enthalpies to the nonlinear regression equation $\theta = \frac{H_{\infty} - H_t}{H_{\infty} - H_0} = e^{-kt^n}$ where θ is crystallinity, H_{∞} , is the levelling-off value of melting enthalpy at which the extent of crystallization in starch stopped, H_t is the melting enthalpy at time t , and H_0 is the melting enthalpy at initial time, t is time of crystallization, k is a rate constant, and n is the Avrami exponent.

Lipid determinations

Flour, dough and bread free lipids (FL).- Flour (10g), ground freeze-dried dough (10g) and ground freeze-dried bread (20g) samples were extracted with light petroleum ether under reflux conditions for 90 min in a soxhlet (McCormack et al., 1991) The solvent was removed under vacuum and the extracts were determined gravimetrically. ~~Determinations were made per duplicate.~~

Flour and dough bound lipids (BL).- Residues of the FL extraction were extracted with chloroform under reflux conditions as described by McCormack et al. (1991) to obtain total bound lipids (non-covalent forces) to both starch and proteins (BL). ~~Determinations were made per duplicate.~~

Bread protein-bound lipids (PBL).- Residues of FL extraction (10g) were treated with 100mL 1% pepsin in 50mM sulphuric acid, (pH 1.6) and gently stirred for 4h at 40°C under the conditions described by Collar et al. (2001). This fraction specifically refers to lipids easily or strongly bound to proteins. ~~Determinations were made per duplicate.~~

Starchy lipids (SL).- Flour and dough starchy lipids were obtained by acid hydrolysis of the non-starchy lipid-free residue (ICC 136). This fraction specifically refers to lipids covalently bound to starch. ~~Determinations were made per duplicate.~~

Bread starch-bound lipids (SBL).- Residues of FL extraction (10g) were reacted with 100mL 0.5% α -amylase in 10mM NaH_2PO_4 , (pH 6.5) and gently stirred for 4h at 70°C. When the reaction was completed, 100mL of Cl_3CH were added, and the mixture stirred for 1h at room temperature and centrifuged.

Supernatants were washed with 5% NaCl, the solvent removed, weighed (SBL) and stored under nitrogen until analysis (Collar et al., 2001). This fraction specifically refers to lipids easily or strongly bound to starch granules either by non-covalent (outside) or covalent forces (inside). ~~Determinations were made per duplicate.~~

Total lipids were indirectly determined by addition of FL+BL+SL amounts retrieved in flours and doughs, and by addition of FL+PBL+SBL levels determined in breads. All lipid fractions and subfractions ~~determinations were made per duplicate~~, and contents were expressed in g/100 g flour basis, as is.

Statistical analysis

Multivariate analysis of variance and correlation matrix of data were performed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN). Multiple range test (Fisher's least significant differences, LSD) for analytical variables was applied to know the difference between each pair of means.

3. Results and discussion

Plural physic-chemical and biochemical approaches have been performed (a) to know the qualitative and quantitative profiles of endogenous lipid fractions of single and multigrain flour, dough and bread matrices and (b) to link lipid binding along breadmaking to the sensory, physical, starch hydrolysis, firming and starch retrogradation kinetics parameters in fresh and aged composite bread matrices.

3.1. Lipid extractability and distribution in single and multigrain flour, dough and bread samples

Data for extractability (g/100 g flour) and distribution (% of total lipids) of lipid fractions and sub-fractions from single and blended flour, dough and bread samples are reported in Table 1 and Figure 1, respectively. Total lipids (g/100 g flour basis) in single flours ranged from 1.85 g (W) to 4.91 g (T), in mixed flours from

1
2
3 213 1.85 g (W) to 2.57 g (111), in doughs from 1.20 g (W) to 2.47 g (101), and in breads from 1.44 g (W) to 3.12
4
5 214 g (101) (Table 1). The total lipid content determined in this study is in general in line with results previously
6
7
8 215 found by Collar et al. (1998, 2001) for wheat, Schloenlechner et al., (2008) for pseudocereals, de Almeida
9
10 216 Costa et al., (2006) for green pea, and Hager et al. (2012) for pseudocereals and teff flours.
11
12 217 Free lipid (FL) was the most prominent fraction in terms of absolute content (Table 1) and as a percentage
13
14 218 of total lipids (Figure 1) in flour (except for GP) and dough samples (except for control W); whereas in
15
16 219 general, protein-bound lipids (PBL) and starch-bound lipids (SBL) predominated in breads with some
17
18 220 exceptions. Bound lipids (BL) were minor lipid fraction in all single and blended flour matrices, while starchy
19
20 221 lipids (SL) accounted for intermediate quantitative lipid fractions in blended flours and doughs (Table 1). FL
21
22 222 in single grain flours varied greatly (from 0.58—GP—to 4.14—T—, g/100 g flour basis, accounting from 28
23
24 223 to 84% of total lipids, respectively), little in blended flours (from 1.33—010—to 1.70—101—, g/100 g flour
25
26 224 basis), doughs (from 0.75—010—to 1.45—101—, g/100 g flour basis, accounting from 44 to 59% of total
27
28 225 lipids) and breads (from 0.42—010—to 0.93—001, 100—, g/100 g flour basis, accounting from 18 to 31-
29
30 226 43% of total lipids). PBL in breads varied from 26 (111) to 47% (010) of total lipids, while SBL accounted
31
32 227 from 25 (001) to 53% (111) of total lipids (Figure 1).
33
34 228 Increased addition of any non-wheat grain flour from 7.5 (0) to 15% (1) significantly changed the quali and
35
36 229 quantitative lipid profile of blended flours, doughs and breads, especially for T and GP (Table 2). At flour
37
38 230 level, blending W flour with increasing amounts of either T or BW promoted the amount of FL by 13-19%
39
40 231 with a concomitant increase of TL by 9-11%, while higher levels of GP induced a decline in FL by 9 % and a
41
42 232 promotion of both BL (12%) and SL (5%) in blended flours, encompassing poorer extractability (-4%) of TL
43
44 233 (Table 2). At dough level, a significant ($p<0.01$) increase of FL (+40%) and BL (+28%) was provided by
45
46 234 blending higher levels of T and GP flours, respectively, which derived in depletion of respective BL (-11%)
47
48 235 and FL (-16%) in composite doughs thereof. At bread stage, GP incorporation at higher levels favoured
49
50 236 accumulation of SBL (+17%) at the expenses of a notable depletion of FL (-40%); whereas blending higher
51
52
53
54
55
56
57
58
59
60

levels of either T or BW resulted in a common decrease in PBL (-13 to -27%) and an accumulation (+29%, T) or depletion (-23%, BW) in SBL (Table 2). The overall result in TL balance was a net increase by 10% in high-T formulated breads, and a net decrease by - 9% (GP) and -14 % (BW), respectively. Simultaneous presence of non-wheat flours at different levels of wheat replacement induced significant interactions on major free and bound lipid subfractions of breadmaking matrices, particularly for T/GP and T/BW (Table 2). Higher levels of T (1) in presence of lower amounts of GP (0) provided maximum extractability (g/100g flour) for FL in blended flours (1.79g), and doughs (1.37g), TL in mixed flours (2.64g) and breads (2.76g), and PBL (0.87g) in breads (Table 2). Whereas, in presence of lower amounts of BW (0), higher T (1) replacement maximized SBL (1.29g) and TL (2.83g) in composite breads. Last results were of the order of those found for the pair GP/BW at 0/0 levels (Table 2).

3.2. Lipid binding during mixing, fermentation and baking of composite matrices

Along breadmaking steps –mixing, fermentation and baking-, lipid subfractions of composite matrices underwent significant quantitative changes as a result of a balance between a) release of protein- and/or starch-bound lipids to the pool of free lipids, b) binding of free lipids to protein and/or starch and c) translocation of protein-bound lipids to starch-bound lipids and/or vice versa. During mixing, a variable depletion of the pool of FL –from -11% (110) to -44% (010, 011)- was denoted in composite matrices, in accordance with a discreet variation in BL –from -17% (100) to +9% (011)- and a moderate promotion of SL –from +8% (100) to +35% (101)- (Figure 2). Increased amounts of either GP or BW from level 0 to level 1 promoted the extent of the changes in FL and minimized those in BL, while increased T replacement led to opposite effects. Control samples on W observed a different lipid pattern during mixing: maximum depletion in FL (-79%) and notable accumulation of both BL (+21%) and SL (+27%). After consecutive fermentation and baking steps of composite matrices, FL decreased from -37% (001) to -72% (111), while a variable sharp increase was denoted for both PBL –from 60% (111) to 236%

(101)- and SBL –from 53% (010) to 175% (101)- (Figure 2). Increasing amounts of any non-wheat flour moderated the changes in PBL but enhanced those of SBL. In absence of non-wheat flours, lipid dynamics of W systems followed a notable decrease in FL (-59%), a moderate increase of PBL (+53%), and a discreet accumulation of SBL (+13%) (Figure 2).

3.3.Relationships between lipid dynamics along breadmaking of composite matrices and physico-chemical, sensory and nutritional properties of fresh and aged breads.

Mixing of composite matrices induced binding from flour to dough of both FL and BL through a respective sharp decrease up to -44% in the pool of free lipids and a slight decrease in BL up to -17%. Concomitantly, a prominent accumulation of SL up to 17% took place in blended doughs (Figure 2). So that, a preferential covalent lipid binding to the inside part of the starch granules takes place during mixing at the expenses of both accessible free lipids and lipids initially bound non-covalently to the gluten/non gluten proteins and to the outside part of the starch granules. Results are in contrast with those found for W systems, where around 80% of FL migrated to the active sites of both gluten and starch leading to a notable accumulation of BL and SL in doughs, respectively (Figure 2). In wheat systems, it has been reported than more than half of the free lipids in flour become associated with the gluten protein during dough mixing (Chung, 1986). The association could involve either physical entrapment or binding of lipids to protein (Carr et al., 1992), and as a result, some of the flour lipids can no longer be extracted with the non-polar solvents used for free lipid extraction. The protein and lipid might be associated through a) chemical interactions involving polar and hydrophobic bonding (Pomeranz and Chung, 1978), suggesting that dough is strengthened by the ability of polar lipids to be associated with protein and starch and/or b) lipid is retained within the gluten in a relatively non-specific way and that the interaction of protein and lipid in gluten may involve the physical entrapment of lipid and also polar or ionic bonding between the components (Carr et al., 1992). It has been alluded that the binding of free lipids with gluten proteins may provide them with the ability to

align at the interface of gas cells during the initial phases of dough mixing and increase gas cell stability throughout the bread making process (Pareyt et al., 2011). When non-gluten forming flours are added to the breadmaking system, interferences in the binding of lipids to main biopolymers -protein, starch- can occur since original wheat flour system is diluted with other fat, protein, starch and dietary fibre entities that compete for water and active sites of biomolecules. This is the case of the present flours used, whose chemical composition is particularly rich in fat (T, BW), protein (GP, BW) and dietary fibre (T, GP, BW). Transformation of dough into bread after successive fermentation and baking steps induced binding of FL and most probably also of BL from dough to bread through a sharp decrease in the pool of FL and a concomitant increase in PBL and SBL of mixed breads (Figure 2). This means that a preferential lipid binding to the gluten/non gluten proteins and to the outside and inside starch granules takes place during fermentation and later baking at the expenses of both a FL displacement and a BL translocation to new protein and starch active sites. Nature of pseudocereal proteins –highly soluble and foaming and emulsifying properties- (Schloenchner et al., 2008), and teff prolamins -lower polymerization, hydrophobicity and denaturation temperature- (Adebowale et al., 2011), can stimulate lipid binding, particularly for the most accessible fraction (FL). In fact, in control W systems, FL migration takes places mainly to the gluten protein active sites and very little to the starch leading to a discreet accumulation of PBL and a poor increase of SBL from dough to bread (Figure 2).

Dynamics on FL and BL during mixing significantly ($p < 0.01$) correlated (coefficient of correlation, r) with some sensory, physical and nutritional features in fresh blended breads: the higher the decrease of FL content over mixing, the higher the cell uniformity ($r = -0.553$), the moistness ($r = -0.761$) and the elasticity ($r = -0.616$) of bread crumb, and the higher the SDS ($r = -0.523$) and RS ($r = -0.530$) formation upon starch hydrolysis; the higher the displacement of BL over mixing, the softer ($r = -0.460$), the more cohesive ($r = -0.593$) and springier ($r = -0.381$) the bread crumb became in blended breads (Table 3).

1
2
3 308 Dynamics on PBL and SBL during fermentation and baking significantly ($p<0.01$) correlated with main
4
5 309 sensory features and physical characteristics in fresh breads, and with firming and retrogradation kinetics
6
7
8 310 on ageing. It can be noticed that the higher the accumulation of PBL over fermentation and baking, the
9
10 311 larger the bread volume ($r=0.453$), and the softer ($r=0.640$) and the more cohesive ($r=0.625$) bread crumbs
11
12 312 are obtained, undergoing slower starch hydrolysis ($r=-0.530$) and retrogradation kinetics ($r=-0.674$) in
13
14 313 blended breads (Table 3). Increased accumulation of SBL after fermentation and baking significantly
15
16 314 impacted the sensory perception of subsequent mixed breads mainly in terms of decreased cell uniformity
17
18 315 ($r=-0.900$), brighter ($r=0.810$), less elastic ($r=-0.768$), but more cohesive ($r=0.755$) fresh bread crumb, with
19
20 316 slower both firming ($r=-0.835$) and starch retrogradation ($r=-0.561$) kinetics (Table 3).
21
22
23
24
25
26

27 318 **4. Conclusions**

28
29 319 Dilution up to 45% of the basic wheat flour matrix by accumulative ternary addition of teff, green pea and
30
31 320 buckwheat flours (7.5%-15% single flour) did significantly ($p<0.01$) impact both the extractability and
32
33 321 distribution of lipid subfractions in composite flours, doughs and breads, and induced dynamics in lipid
34
35 322 binding over mixing, fermentation and baking steps, in variable extent. At dough level, a significant
36
37 323 promotion of FL or BL was provided by blending higher levels of T or GP flours, respectively, which
38
39 324 encompassed a depletion of respective BL or FL in composite doughs thereof. At bread stage, GP
40
41 325 incorporation at higher levels favoured accumulation of SBL at the expenses of a notable depletion of FL,
42
43 326 whereas blending larger amounts of either T or BW resulted in a common decrease in PBL and an
44
45 327 accumulation (T) or depletion (BW) in SBL.
46
47
48
49

50 328 During mixing, a preferential covalent lipid binding to the inside part of the starch granules takes place at the
51
52 329 expenses of both accessible free lipids and lipids initially bound non-covalently to the gluten/non gluten
53
54 330 proteins and to the outside part of the starch granules in flours. Results are in contrast with those found for
55
56 331 W systems, where around 80% of FL migrated to the active sites of both gluten and starch leading to a
57
58
59
60

notable accumulation of BL and SL in doughs, respectively. Higher depletion in FL contents over mixing corresponded to more uniform cell structure, moist and elastic bread crumbs, exhibiting larger formation of both SDS and RS upon starch hydrolysis. Concomitantly, the higher the displacement of BL over mixing, the softer, the more cohesive and springier the bread crumb was in blended breads. During fermentation and later baking a preferential lipid binding to the gluten/non gluten proteins and to the outside and inside starch granules takes place at the expenses of both a FL displacement and a BL translocation to new protein and starch active sites. It can be noticed that the higher the accumulation of PBL over fermentation and baking, the larger the bread volume, and the softer and the more cohesive bread crumbs are obtained, undergoing slower starch hydrolysis and retrogradation kinetics in composite breads. Increased accumulation of SBL after fermentation and baking significantly impacted the sensory perception of subsequent mixed breads mainly in terms of decreased cell uniformity, brighter, less elastic, but more cohesive fresh bread crumbs, with slower both firming and starch retrogradation kinetics.

5. Acknowledgements

The authors acknowledge the financial support of Consejo Superior de Investigaciones Científicas (CSIC), Ministerio de Economía y Competitividad (MINECO) and Fondo Europeo de Desarrollo Nacional (FEDER) (Project AGL2015-63849-C2-1-R).

6. References

- AACC 2005. Approved methods of the American Association of Cereal Chemists, AA CC. 10th edition, St. Paul, Minn.
- Adebowale A. A., Emmambux, M. N., Beukes, M., & Taylor, J. R. N. (2011). Fractionation and characterization of teff proteins. *Journal of Cereal Science*, 54, 380-386.

1
2
3 355 Angioloni, A., & Collar, C. (2011a). Significance of lipid binding on the functional and nutritional profiles of
4
5 356 single and multigrain matrices. *European Food Research and Technology*, 233,141–150.
6
7
8 357 Angioloni, A., & Collar, C. (2011b). Physicochemical and nutritional properties of reduced-caloric density
9
10 358 high-fibre breads. *LWT - Food Science and Technology*, 44, 747-758.
11
12 359 AOAC. (2000). Official Methods of Analysis 17th Ed., AOAC International.
13
14
15 360 Armero, E., & Collar, C. (1998). Crumb firming kinetics of wheat breads with antistaling additives. *Journal of*
16
17 361 *Cereal Science*, 28, 165-174.
18
19 362 Carr, N. O., Daniels, N. W. R., & Frazier, P. J. (1992). Lipids interactions in breadmaking. *Critical Reviews*
20
21 363 *in Food Science and Nutrition*, 31(3), 237–258.
22
23
24 364 Chung, O. K. (1986). Lipids–protein interactions in wheat flour, dough, gluten, and protein fractions. *Cereal*
25
26 365 *Foods World*, 31(3), 242–256.
27
28
29 366 Collar, C., & Angioloni, A. (2014). Pseudocereals and teff in complex breadmaking matrices: impact of lipid
30
31 367 dynamics on the bread functional and nutritional profiles. *Journal of Cereal Science*, 59, 145-154.
32
33 368 Collar, C., Bollain, C., & Angioloni, A. (2005). Significance of microbial transglutaminase on the sensory,
34
35 369 mechanical and crumb grain pattern of enzyme supplemented fresh pan breads. *Journal of Food*
36
37 370 *Engineering*, 70, 479–488.
38
39
40 371 Collar, C., Jiménez, T., Conte, P., & Fadda, C. (2014a). Impact of ancient cereals, pseudocereals and
41
42 372 legumes on starch hydrolysis and antiradical activity of technologically viable blended breads.
43
44 373 *Carbohydrate Polymers*, 113, 149–158.
45
46
47 374 Collar, C., Jiménez, T., Conte, P., & Piga, A. (2015). Significance of thermal transitions on starch
48
49 375 digestibility and firming kinetics of restricted water mixed flour bread matrices. *Carbohydrate Polymers*,
50
51 376 122, 169–179.
52
53
54 377 Collar, C., Armero, E., & Martínez, J. (1998). Lipid binding of formula bread doughs Relationships with
55
56 378 dough and bread technological performance. *Z. Lebensm Unters Forsch A*, 207, 110–121.
57
58
59
60

- 379 Collar, C., Martínez, J. C., & Rosell, C. M. (2001). Lipid Binding of Fresh and Stored Formulated Wheat
380 Breads. Relationships with Dough and Bread Technological Performance. *Food Science and*
381 *Technology International*, 7/6, 501–510.
- 382 de Almeida Costa G.E., da Silva Queiroz-Monici K., Reis A.M.P.M., & de Oliveira A.C. (2006). Chemical
383 composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean,
384 chickpea and lentil legumes. *Food Chemistry*, 94, 327–330.
- 385 Gan, Z., Ellis, P.R., & Schofield, J.D. (1995). Gas cell stabilization and gas retention in wheat bread dough.
386 *Journal of Cereal Science* 21, 215-230.
- 387 Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat
388 flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food*
389 *Science and Technology*, 16, 12–30.
- 390 Hager, A. S., Wolter, A. Jacob, F., Zannini, E., & Arendt, E. K. (2012). Nutritional properties and ultra-
391 structure of commercial gluten free flours from different botanical sources compared to wheat flours.
392 *Journal of Cereal Science*, 56, 239-247.
- 393 ICC, 1976-1996. ICC Standard Methods of the International Association for Cereal Chemistry. 104/1, 105/2,
394 110/1, 115/1, 136, 162, 166. The Association: Vienna.
- 395 McCann, T. H., Small, D. M., Batey, I. L., Wrigley, C. W., & Day, L. (2009). Protein–lipid interactions in
396 gluten elucidated using acetic-acid fractionation. *Food Chemistry* 115, 105–112.
- 397 McCormack G., Panozzo J., Bekes, F., & MacRitchie F. (1991). Contributions to breadmaking of inherent
398 variations in lipid content and composition of wheat cultivars. I. Results of survey. *Journal of Cereal*
399 *Science*, 13, 255–261.
- 400 Pareyt, B., Finnie, S. M., Putseys, J. A., & Delcour, J. A. (2011). Lipids in bread making: Sources,
401 interactions, and impact on bread quality. *Journal of Cereal Science*, 54, 266-279.

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

402 Pomeranz, Y., & Chung, O. K. (1978). Interaction of lipids with proteins and carbohydrate in breadmaking.
403 *Journal of the American Oil Chemists' Society*, 55, 285–289.

404 Putseys, J.A., Lamberts, L., & Delcour, J.A. (2010). Amylose-inclusion complexes: Formation, identity and
405 physico-chemical properties. *Journal of Cereal Science*, 51, 238-247.

406 Schoenlechner, R., Siebenhandl, S., & Berghofer, E. (2008). Pseudocereals. In: Arendt, E.K., Dal Bello, F.
407 Eds., *Gluten-free Cereal Products and Beverages*, Ireland, Cork, pp. 149–176.

408 Selmair, P.L., & Koehler, P. (2010). Role of glycolipids in breadmaking. *Lipid Technology* 22, 7-10.

409 Setser, C.S. (1996). Sensory methods. In: *Baked goods freshness*, R.E. Hebda, H.F. Zobel (Eds.), Marcel
410 Decker Inc, New York (1996), pp. 171–187.

411 Sroan, B., & MacRitchie, F. (2009). Mechanism of gas cell stabilization in breadmaking. II. The secondary
412 liquid lamellae. *Journal of Cereal Science*, 49, 41-46.

Table 1 .- Lipid subfractions (g/100 g of flour) from single flours and blended doughs and breads (mean values of three replicates $\pm$ standard deviation).

Code ¹	Free lipids			Bound lipids		Protein-bound	Starchy lipids		Starch bound	Total Lipids		
	Flour	Dough	Bread	Flour	Dough	Bread	Flour	Dough	Bread	Flour	Dough	Bread
010	1.33 $\pm$ 0.09	0.75 $\pm$ 0.08	0.42 $\pm$ 0.08	0.37 $\pm$ 0.06	0.35 $\pm$ 0.09	1.09 $\pm$ 0.09	0.54 $\pm$ 0.08	0.62 $\pm$ 0.04	0.82 $\pm$ 0.12	2.23	1.72	2.33
001	1.47 $\pm$ 0.11	1.13 $\pm$ 0.09	0.93 $\pm$ 0.07	0.33 $\pm$ 0.05	0.31 $\pm$ 0.07	0.70 $\pm$ 0.09	0.52 $\pm$ 0.06	0.60 $\pm$ 0.09	0.54 $\pm$ 0.09	2.33	2.04	2.17
011	1.44 $\pm$ 0.13	0.82 $\pm$ 0.10	0.49 $\pm$ 0.10	0.37 $\pm$ 0.07	0.40 $\pm$ 0.10	0.80 $\pm$ 0.12	0.54 $\pm$ 0.09	0.61 $\pm$ 0.06	0.97 $\pm$ 0.12	2.34	1.84	2.26
000	1.37 $\pm$ 0.08	1.08 $\pm$ 0.12	0.51 $\pm$ 0.11	0.32 $\pm$ 0.08	0.32 $\pm$ 0.11	1.02 $\pm$ 0.10	0.52 $\pm$ 0.05	0.67 $\pm$ 0.12	1.05 $\pm$ 0.13	2.21	2.07	2.58
111	1.67 $\pm$ 0.12	1.33 $\pm$ 0.09	0.47 $\pm$ 0.09	0.37 $\pm$ 0.02	0.36 $\pm$ 0.09	0.59 $\pm$ 0.11	0.54 $\pm$ 0.03	0.66 $\pm$ 0.09	1.18 $\pm$ 0.10	2.57	2.36	2.24
101	1.70 $\pm$ 0.11	1.45 $\pm$ 0.08	0.59 $\pm$ 0.09	0.33 $\pm$ 0.05	0.32 $\pm$ 0.08	1.10 $\pm$ 0.11	0.52 $\pm$ 0.10	0.70 $\pm$ 0.10	1.44 $\pm$ 0.09	2.55	2.47	3.12
100	1.60 $\pm$ 0.07	1.21 $\pm$ 0.13	0.93 $\pm$ 0.08	0.32 $\pm$ 0.10	0.27 $\pm$ 0.08	0.86 $\pm$ 0.08	0.52 $\pm$ 0.09	0.56 $\pm$ 0.11	1.26 $\pm$ 0.09	2.44	2.04	3.06
110	1.39 $\pm$ 0.08	1.24 $\pm$ 0.12	0.56 $\pm$ 0.11	0.37 $\pm$ 0.09	0.35 $\pm$ 0.07	0.79 $\pm$ 0.07	0.53 $\pm$ 0.08	0.65 $\pm$ 0.09	1.22 $\pm$ 0.08	2.29	2.24	2.57
Control	1.06 $\pm$ 0.10	0.22 $\pm$ 0.06	0.43 $\pm$ 0.09	0.28 $\pm$ 0.11	0.34 $\pm$ 0.12	0.43 $\pm$ 0.02	0.51 $\pm$ 0.10	0.65 $\pm$ 0.06	0.57 $\pm$ 0.11	1.85	1.20	1.44
Flours ²												
W	1.06b			0.28a			0.51a			1.85		
T	4.14d			0.28a			0.48a			4.91		
GP	0.58a			0.83b			0.68b			2.09		
BW	2.50c			0.32a			0.54a			3.36		

¹ Blended samples were based on: wheat (W), teff (T), green pea (GP), and buckwheat (BW). Three digit sample code refers to low (0) and high (1) wheat flour replacement by T: GP: BW flours in sample formulation.

² Within each column of single flour samples, values with different letters differ significantly from each other ($p < 0.05$).

Table 2.- Significant (p<0.01, **) single and 2nd order interactive effects of non-wheat flours -teff (T), green pea (GP), buckwheat (BW)- on lipid subfractions contents -free lipids (FL), bound lipids (BL), starchy lipids (SL), protein bound lipids (PBL) and starch-bound lipids (SBL) in composite matrices.

Parameter, (g/ 100 g flour)	Single effects ¹							2nd order Interactions ¹							
	Level ²	Overall mean	T	**	GP	**	BW	**	Level ²	T * GP	**	T * BW	**	GP * BW	**
FL Flour	0	1.53	1.40±0.03	a	1.60±0.05	b	1.44±0.03	a	0 0	1.42±0.04	a	1.35±0.04	a	ns	
	1		1.66±0.05	b	1.46±0.03	a	1.63±0.05	b	0 1	1.39±0.04	a	1.46±0.04	b		
									1 0	1.79±0.08	c	1.52±0.04	c		
									1 1	1.53±0.04	b	1.80±0.09	d		
BL Flour	0	0.35	ns		0.33±0.004	a	0.347±0.002	a	0 0	ns		ns		0.32±0.003	a
	1				0.37±0.003	b	0.35±0.005	b	0 1					0.33±0.004	b
									1 0					0.37±0.003	c
									1 1					0.37±0.003	c
TL Flour	0	2.41	2.28±0.03	a	2.46±0.05	b	2.31±0.03	a	0 0	2.27±0.03	a	2.22±0.03	a	ns	
	1		2.53±0.05	b	2.36±0.03	a	2.51±0.04	b	0 1	2.285±0.03	a	2.34±0.03	b		
									1 0	2.64±0.06	c	2.40±0.03	b		
									1 1	2.43±0.03	b	2.68±0.05	c		
FL Dough	0	1.14	0.94±0.07	a	1.24±0.11	b	ns		0 0	1.11±0.07	b	ns		ns	
	1		1.33±0.11	b	1.04±0.07	a			0 1	0.79±0.07	a				
									1 0	1.37±0.14	c				
									1 1	1.29±0.07	c				
BL Dough	0	0.33	0.35±0.01	b	0.29±0.02	a	ns		0 0	ns		ns		0.31±0.015	a
	1		0.31±0.02	a	0.37±0.02	b			0 1					0.28±0.02	a
									1 0					0.35±0.014	b
									1 1					0.38±0.014	c
FL bread	0	0.65	ns		0.81±0.16	b	ns		0 0	ns		ns		0.64±0.11	a
	1				0.49±0.1	a			0 1					0.98±0.16	b
									1 0					0.49±0.1	a
									1 1					0.48±0.1	a
PBL Bread	0	0.84	0.90±0.07	b	ns		0.97±0.07	b	0 0	0.86±0.07	b				
	1		0.78±0.11	a			0.71±0.10	a	0 1	0.95±0.07	c				
									1 0	0.87±0.14	abc				
									1 1	0.69±0.07	a				
SBL Bread	0	0.97	0.85±0.05	a	0.9±0.08	a	1.11±0.05	b	0 0	ns		0.94±0.05	b	1.2±0.06	c
	1		1.1±0.08	b	1.05±0.05	b	0.85±0.04	a	0 1			0.76±0.05	a	0.60±0.08	a
									1 0			1.29±0.06	c	1.02±0.05	b
									1 1			0.92±0.08	b	1.08±0.05	b
TL Bread	0	2.46	2.34±0.02	a	2.57±0.03	b	2.64±0.02	b	0 0	2.38±0.02	b	2.46±0.02	c	2.84±0.02	c
	1		2.58±0.03	b	2.35±0.02	a	2.27±0.03	a	0 1	2.30±0.02	a	2.22±0.02	a	2.30±0.03	a
									1 0	2.76±0.04	c	2.83±0.02	d	2.45±0.02	b
									1 1	2.41±0.02	b	2.33±0.03	b	2.25±0.02	a

¹ Mean values ± standard deviation. Within each parameter, values with different letters differ significantly from each other.

² Coded levels refer to 7.5% (0) and 15% (1) wheat flour replacement by non-wheat flours in sample formulation.

Table 3.- Pearson correlations ($p < 0.01$) between dynamics in lipid subfractions along breadmaking and sensory, physical, starch hydrolysis, firming and starch retrogradation kinetics parameters of composite matrices.

	Parameters	FL _{fb}	PBL _{fb}	SBL _{fb}	FL _{mixing}	BL _{mixing}	SL _{mixing}
Sensory Evaluation	cell uniformity			-0.8999	-0.5531		-0.3899
	cell size			-0.7689			
	brightness			0.8101	0.6801	-0.4999	
	moistness			-0.8056	-0.7616	0.4819	
	elasticity			-0.7679	-0.6164		-0.5134
	smoothness			-0.5955		0.5310	
	mouthfeel			0.8101	0.6801	-0.4999	
	adhesiveness			-0.5130		0.5966	
	cohesiveness			0.7553	0.7868	-0.3933	
	chewiness			0.7627	0.8081		0.4358
Physical Characteristics	Specific volume		0.4533		0.4738		0.6346
	Hardness		-0.6398		-0.6053	0.4569	-0.3810
	Cohesiveness	0.6931	0.6247			-0.5931	
	Springiness					-0.3805	
	Crumb whiteness	0.3994	0.5336			-0.4174	
	Cell density			-0.6074	-0.6577		-0.7097
Starch Digestibility	C_{∞}	-0.4675	0.4634	0.7548			
	k_d		-0.5298		0.4543		
	RDS					-0.5742	
	SDS				-0.5234		
	RS			-0.5372	-0.5298	0.5449	
Firming kinetics	T_{∞}			0.5912	0.5895	-0.6217	
	k_f			-0.8353	-0.5777		-0.4263
	n_f	-0.5041				0.5461	0.4678
	T_0		-0.6686	-0.3521	-0.5707	0.4032	-0.3945
Amylopectin Retrogradation kinetics	ΔH_{∞}		0.6016	0.6365			
	k_r		0.3928				
	n_r		-0.6741	-0.5608	-0.3852	0.4230	

FL_{fb}: changes in free lipids during fermentation and baking, PBL_{fb}: changes in protein-bound lipids during fermentation and baking, SBL_{fb}: changes in starch-bound lipids during fermentation and baking, FL_{mixing}: changes in free lipids during mixing, BL_{mixing}: changes in bound lipids during mixing, SL_{mixing}: changes in starchy lipids during mixing, C_{∞} maximum starch hydrolysis extent, k_d kinetic constant for starch hydrolysis, RDS rapidly digestible starch, SDS slowly digestible starch, RS resistant starch, T_{∞} crumb firmness at time ∞ , k_f constant of proportion of retrogradation kinetics, n_f Avrami exponent of firming kinetics, T_0 crumb firmness at time 0, ΔH_{∞} retrogradation enthalpy at ∞ , k_r constant of proportion of firming kinetics, n_r Avrami exponent of retrogradation kinetics.

(A)

(B)

(C)

Figure 1.- Lipid distribution (%) in flour (A), dough (B) and bread (C) samples. Blended samples were based on: wheat (W), teff (T), green pea (GP), and buckwheat (BW). Three digit bread sample code refers to low (0) and high (1) wheat flour replacement by T: GP: BW flours in sample formulation. FL: free lipids, BL: bound lipids, SL: starchy lipids, PBL: protein-bound lipids, SBL: starch-bound lipids, TL: total lipids.

210x297mm (180 x 180 DPI)

(A)

(B)

Figure 2.- Changes (%) in lipid subfractions -free lipids (FL), bound lipids (BL), starch lipids (SL) during mixing (A), and in free lipids (FL), protein bound lipids (PBL), starch bound lipids (SBL) during fermentation and baking (B). Blended samples were based on: wheat (W), teff (T), green pea (GP), and buckwheat (BW). Three digit bread sample code refers to low (0) and high (1) wheat flour replacement by T: GP: BW flours in sample formulation.

210x297mm (200 x 200 DPI)