Pseudocereals and teff in complex breadmaking matrices: impact on lipid dynamics.

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Abstract The use of pseudocereals and ancient grains for breadmaking applications is receiving particular attention since they involve nutrient dense grains with proven with health-promoting attributes. Dilution up to 20% of the basic rye/wheat flour blend by accumulative addition of amaranth, buckwheat, quinoa and teff flours (5% single flour) did positively impact either some dough visco-metric and visco-elastic features, or some techno-functional and nutritional characteristics of mixed bread matrices, and induced concomitant dynamics in lipid binding over mixing and baking steps. A preferential lipid binding to the gluten/non gluten proteins and to the outside part of the starch granules takes place during mixing, in such a way that the higher the accumulation of bound lipids over mixing, the higher the bioaccessible polyphenol content in blended breads. During baking, lipids bind to the gluten/non gluten proteins at the expenses of both a free lipid displacement and a lipid migration from the inside part of the starch granules to the protein active sites. It was observed that the higher the decrease of free lipid content over baking, the higher the pasting temperature and the lower the total setback on cooling and the dynamic moduli, but the higher the specific volume in blended breads.

Introduction

Revisiting under-utilized plant species such as pseudocereals and ancient grains for breadmaking applications arises from the finding and promotion of nutritionally and health-related relevant attributes. Their innovation is rather related to the ways in which old and new uses are being readdressed, since pseudocereals and ancient grains have been used by local populations in traditional ways for many centuries (Dini et al., 2012). Pseudo-cereal flours with some nutritional and functional features preferable to cereal flours (Fessas et al., 2008), can be excellent sources of proteins, vitamins, minerals, fiber, and other important nutrients (Coda et al., 2012), and show antioxidant, antinflammatory, and anticarcinogenic activities (Lin et al., 2008). Pseudocereal proteins are highly soluble and characterized by foaming and emulsifying properties (Schoenlechner et al., 2008). The amino acid profile of the proteins of amaranth is comparable to that of egg, and the nutritional quality of the proteins of quinoa is comparable to that of caseins (Schoenlechner et al., 2008). Compared to cereals, quinoa has a higher concentration of fat with elevated levels of unsaturated fatty acids and phospholipids which, due to the presence of vitamin E, remain stable during storage (Ng et al., 2007). In addition, quinoa shows a balanced aminoacid spectrum with high methionine and lysine contents (Peiretti et al., 2013). Teff (*Eragrostis tef*) is a nutritious cereal grain indigenous to Ethiopia, rich in carbohydrate and fibre, that contains more iron, calcium and zinc than other cereal grains, including wheat, barley and sorghum (Abebe et al., 2007), and constitutes a promising basic ingredient for achieving healthy cereal products (Alaunyte et al., 2012).

The nutritional properties and baking characteristics of amaranth, quinoa and buckwheat have been assessed in gluten-free matrices (Alvarez-Jubete et al., 2010), achieving breads with superior nutritional features and acceptable sensory scores. In wheat flour matrices, some studies demonstrated the feasibility of partial/low replacement of wheat flour with pseudocereals for

processing baked goods (Tosi et al., 2002; Schoenlechner et al., 2008; Angioloni and Collar, 2011a, b). The use of a blend of buckwheat, amaranth, chickpea and quinoa flours subjected to sourdough fermentation by selected γ-aminobutyric acid (GABA)-producing strains allowed the manufacture of a bread enriched of GABA and should be considered as a promising possibility for enhancing nutritional, functional, sensory, and technological properties of bread. The addition of quinoa and/or buckwheat seeds (at levels of 30 and 40%) previously subjected to an hydrothermal process, resulted in a valuable effect on the nutritive value of the breads (Demin et al., 2013). Teff flour, despite being gluten-free, has been reported to produce high-quality leavened flatbread aging much slower than if made from other cereals, in particular sorghum (*Sorghum bicolor* (L.) Moench) (Taylor and Emmambux, 2008). Replacement of up to 30% of wheat flour by teff flour in presence of a mixture of amylolytic and non amylolytic enzymes can lead to acceptable breads (Alaunyte et al., 2012).

Pseudocereals and teff flours exhibit higher quali and quantitative lipid profiles than wheat flours do (Hager et al., 2012). 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). In breadmaking applications, protein and starch lipid binding in wheat flour and bread systems have been reported to correlate with loaf volume, crumb structure, softness and/or texture of bread (Collar et al., 2001, 2011). 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 bound 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). In single and blended oat, rye, buckwheat and wheat flour matrices, lipids bound to proteins during dough mixing

are translocated and bound to starch during baking. Starch lipid showed the most significant correlations with parameters related to dough and bread performance during breadmaking, especially over the mixing step (Angioloni and Collar, 2011).

This research is aimed at characterising the lipid fractions at flour, dough and bread stages of single and blended amaranth, buckwheat, quinoa and teff added to a wheat/rye matrix, prior to analyse the significance of starch- and protein-lipid binding on the functional and nutritional properties of associated grain matrices along mixing and baking.

Experimental

Materials

Commercial flours from refined (70% extraction rate) common Wheat *Triticum aestivum* (W), and whole Rye *Secale cereale* (R), Amaranth *Amaranthus caudatus* – (A), Buckwheat *Fagopyrum esculentum* (B), Quinoa *Chenopodium quinoa* (Q) and Teff *Eragrostis tef* (T) were purchased from the Spanish market. Ireks *Vollsauer* sour dough was from Ireks (Spain); commercial compressed yeast was from Lesaffre (France); Novamyl 10000 a maltogenic intermediate thermostable alpha amylase was purchased from Novozymes (Denmark).

2.2. Methods

Chemical,functional and nutritional composition of flours

Moisture, protein, ash and fat contents of commercial flours W, R, T, A, B and Q were determined following the ICC methods 110/1, 105/2, 104/1, and 136, respectively (ICC, 1976-1996). Total, soluble and insoluble dietary fibre contents were determined according to the AOAC method

991.43 (AOAC, 1991).Two replicates were made for each flour analysis. Digestible carbohydrates were calculated by difference (FAO, 2003). Solvent-Retention Capacity (SRC) was determined according the AACC method 56-11 (AACC, 2005). The Water-Holding Capacity (WHC) was determined as described by Traynham et al., 2007. Fat adsorption capacity (FAC) was determined according to Ahn et al. (2005). Foam capacity (FC) and Foam stability (FS) were determined as described by Alu'datt et al (2012).

Bread making of blended flours

Doughs and breads were prepared for a) control (W-R, 50:50, wt:wt), b) singly added A, B, Q and T at 5% W-R flour basis, respectively, c) binary added QA, QB, QT, AB, AT, and BT at 10% (5%+5%) W-R flour basis, respectively, d) ternary added QAB, QAT, ABT, and QBT at 15% (5%+5%+5%) W-R flour basis, respectively, and quaternary added QABT at 20% (5%+5%+5%+5%) W-R flour basis, respectively. 16 different blended flours were obtained. Blended flour, water (88% -WR-, 89% -Q-, 90% -A-, 91% -B- and 92% -T-, flour basis), commercial compressed yeast (4% flour basis), salt (1.5% flour basis), sugar (2% flour basis), commercial sour dough (10% flour basis), skimmed milk powder (5%, flour basis), Novamyl (7.5 mg, flour basis) and calcium propionate (0.5%) were mixed in a 10 kg mixer at 60 revolutions min-1 for 10 min up to optimum dough development. Fermented doughs were obtained after bulk fermentation (10 min), dividing (300 g), rounding, molding, and proofing up to maximum volume increment (30 min), and were baked at 200 ºC for 30 min to make control, and pseudocereal- and teff- enriched breads. Breads were sliced (2 cm) and stored in polypropylene bags for 1, 3, 6, 8 and 10 days at 22ºC until analysis.

Dough functionality

Dough functional behaviour was assessed by either fundamental or empirical dough physical tests. Dough viscoelasticity was determined by dynamic oscillation tests on an RS1 controlled stress rheometer equipped with a Phoenix II circulating bath (Haake, Karlsruhe, Germany) using a 60-mm serrated plate–plate geometry with a 1-mm gap between plates (Angioloni and Collar, 2012a). Strain sweep tests were run to identify the linear viscoelastic region. Oscillatory measurements of storage modulus (G') and loss modulus (G'') were performed at 25 º C within a frequency range from 0.1 to 10 Hz. All measurements were made in triplicate. Viscometric properties -dough pasting profiles (gelatinization, pasting, and setback properties)- were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC standard method 162 (Collar, 2003). RVA parameters were calculated from the pasting curve using Thermocline v. 2.2 software.

Bread measurements

Physico-chemical and sensory determinations

Specific volume was assessed by seed displacement, and aspect ratio was calculated as width/height ratio of central slides. Colour determinations were carried out on bread crumb and crust using a Minolta colorimeter (Minolta CR- 400, Konica Minolta Sensing, Inc., Osaka, Japan), and results were expressed in accordance with the Hunter Lab colour space. Parameters determined were L (L = 0 [black] and L = 100 [white]), a (-a = greenness and +a = redness), and b (-b = blueness and +b = yellowness). Sensory analysis of fresh breads was carried out by a consumer acceptability test. Overall acceptability was tested by a group of 30 consumers using a 9 point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). Bread primary and secondary mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems) using a 10 mm diameter probe, a 5 kg load cell, 50% penetration depth and a 30 s gap between compressions on

slices of 20 mm width. For textural measurements, three slices of two breads were used for each sample at different storage periods (0 to 10 days). The obtained firming curves were modelled using the Avrami equation (Armero and Collar, 1998).

Nutritional determinations

Chemical and nutritional composition.- Moisture, protein, ash, fat, total, soluble and insoluble dietary fibre contents and digestible carbohydrates of fresh breads were determined following the sample methodology reported for flours.Two replicates were made for each analysis.

Polyphenol content and antiradical activity.- Total, free and bound polyphenol content was determined according to the Folin-Ciocalteu procedure, as previously reported by Angioloni and Collar (2011a) in extracts isolated as described earlier by Angioloni and Collar, 2012b. Total flavonoid content was determined as described by Angioloni and Collar, 2012b. For the determination of bioaccessible phenols, bread samples were processed by an *in vitro* digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract, skipping the colonic fermentation in the large intestine, according to the procedure of Glahn et al. (1998) and used by Angioloni and Collar (2011a) for multigrain breads. The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was used to measure the antiradical activity of the samples according to the DPPH• method and applied earlier (Angioloni and Collar, 2011a). Bioaccessible phenol determination was conducted in bread samples by conducting an “in vitro” digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract as used by Angioloni and Collar (2011a) for breads.

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.*

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).

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 40ºC under the conditions described by Collar et al. (2001). This fraction specifically refers to lipids easily or strongly bound to proteins.

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.

Bread starch-bound lipids (SBL).- Residues of FL extraction (10g) were reacted with 100mL 0.5% α-amylase in 10mM NaH2PO4, (pH 6.5) and gently stirred for 4h at 70ºC. When the reaction was completed, 100mL of Cl3CH 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. This fraction specifically refers to lipids easily or strongly bound to starch granules either by non-covalent (outside) or covalent forces (inside).

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 contents were expressed in g/100 g flour basis, as is.

Statistical analysis

Multivariate (MANOVA, non linear multiple regression) analysis of data was performed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN).

Results and discussion

Chemical, functional and nutritional performance of single flours.

The chemical, functional and nutritional profiles of amaranth, quinoa, buckwheat and teff flours *vs* rye and refined wheat flours (Table 1) evidenced marked differences in chemical and nutritional component levels (per 100 g flour, m. b.) within milled grains. Moisture content of flours ranged from 11.89 (teff) to 14.32 % (wheat), fat content was notably higher in pseudocereals, particularly amaranth (5.08%) and teff (4.46%) than in rye (0.93%) and wheat (1.34%) flours, and total dietary fibre content of pseudocereals, rye and teff was from 5 (teff) to 7 times (quinoa) the level found in refined wheat flour (2.19%). Except for buckwheat flour (13.07%), the level of protein of pseudocereals, quinoa and amaranth, and teff was similar (around 11%), lower than proteins in wheat flour (12.11%), and much higher than proteins found in rye flour (8.92%). On the contrary, digestible carbohydrates of pseudocereals and teff flours that ranged 56-59%, were inferior to the amount found for rye (64%) and wheat flours (70%). A favourable chemical composition of amaranth, quinoa, buckwheat and teff flours has been underlined with respect to wheat and/or rye flours (Hager et al., 2012).

Hydration properties (WHC and SRC), FAC, FC and FS showed different pattern depending on the grain flour (Table 1). WHC that reports the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary, and physically entrapped water against gravity followed the general order amaranth, quinoa, teff > rye, buckwheat > wheat, probably ascribed to the formation of large clusters of protein molecules or protein aggregates bound by hydrogen bounds and other non-covalent forces in pseudocereal and ancient grains. SRC testing used to establish a practical functionality profile of flour (Heywood et al., 2002), takes into account several flour constituents influencing water-retention potential, including pentosans, damaged starch, and glutenin, using

sucrose, sodium carbonate, and lactic acid solutions, respectively. For flour typically used to produce bread by the sponge–dough method, optimal SRC profile values would be ≥100% glutenin, ≤96% pentosans, ≤72% damaged starch (Heywood et al., 2002). According to this, except wheat flour grain flours, pseudocereals and teff hardly fit the water retention profile for bread flours as it can be expected from the lack of gluten proteins. FAC values that indicate the ability of protein to bind fat depend on nonpolar side chains that bind hydrocarbon chains, thereby contributing to increased oil absorption (Ahn et al., 2005). Higher FAC values for amaranth and rye flours can be partly attributed to a marked decrease in bulk density because fat absorption depends on the physical entrapment of oil. Both FC and FS of grain flours differed greatly (Table 1). Rye flour exhibited superior FC (26 mL) than wheat (14 mL) and the other flours (3-9 mL), and high FS (81-96%), similar to buckwheat and quinoa flours (100%).

Plural physic-chemical and biochemical approaches have been performed (a) to assess the functional and nutritional pattern of single and multigrain dough and bread matrices, (b) to know the qualitative and quantitative profiles of endogenous lipid fractions and (c) to link lipid binding in flour, dough and bread to the functional and nutritional features of single and blended matrices.

Physic-chemical properties of single and multigrain doughs and functional and nutritional properties of fresh breads

Individual data for visco-metric and visco-elastic properties of single and multigrain doughs, techno-functional and nutritional properties of fresh breads and staling/keeping behaviour of stored breads are reported in Table 2. The measured parameters indicate that in general, there is a moderate variation amongst the tested samples in consequence of the different grain flours used (Table 3). In fact, dilution up to 20% of the basic rye/wheat flour blend by accumulative addition of amaranth, buckwheat, quinoa and teff flours (5% single flour) did not lead to significant differences ($p<0.01$)

either in some dough visco-metric features, or in some techno-functional, nutritional and keeping characteristics of mixed bread matrices. Peak viscosity (1572-1701cP), holding strength (862-930cP), viscosity of the hot paste (976-1142cP), and viscosity of the cold gel (1366-1452cP) did not depend on the grain flour addition (Table 2). Analogously, characteristics of fresh -overall acceptability (4-7/10), aspect ratio (1.5-1.8), springiness (0.94-0.99), lightness (57-61), fat (2.93-3.09%), protein (8.20-8.80%), digestible carbohydrates (43-45%), free phenolics (256-365mg/100g bread, as is), and flavonoids (264-425mg/100g bread, as is)- and stored $-T_0$ (5.07-9.42 N), *n* (0.46-1.48), and *k* (0.08-0.43)- mixed breads were not derived from the added grain flours (Table 2). On the contrary, single presence of amaranth, buckwheat, quinoa and/or teff significantly affect the extent of some visco-metric, visco-elastic, physic-chemical and nutritional parameters of blended breads (Table 3). No significant second order interactions have been retrieved on the above mentioned parameters, so that only additive effects were observed from the multiple addition of non-wheat grain flours to the wheat-rye basic matrix. Single addition of any of the non-wheat flours significantly promoted dietary fibre content, both soluble and insoluble fractions by 6% in breads thereof (Table 3), in good accordance with the high fibre content of grain flours (Table 1). Except for buckwheat flour, individual grain flours promoted the gelling viscosity profile during cooling (+9% total setback, +4% final viscosity) and the dynamic moduli values by 20% (G', G''). Additionally, quinoa addition delayed the pasting temperature by 1 %, and decreased the breakdown on cooking by 5 % and the bioaccessible polyphenol content by 6 % (Table 3). Single incorporation of buckwheat flour at 5%, wheat-rye basis only impacted some physic-chemical and nutritional measured characteristics of fresh breads: cohesiveness and resilience underwent a decrease by 3 and 6%, respectively; whereas antiradical activity was promoted by 15 % (Table 3). Single amaranth provided lower volume breads (-8%), with harder texture (+20) at long term-storage, but higher content of bioaccessible polyphenols (+6%). The presence of teff flour decreased bread volume by 8%.

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 4 and Fig. 1, respectively. Total lipids (g/100 g flour basis) in single flours ranged from 1.85 g (wheat) to 5.81 g (amaranth), in mixed flours from 1.76 g (W-R) to 2.22 g (W-R-Q-A-B-T), in doughs from 1.24 g (W-R-B) to 1.85 g (W-R-A-T), and in breads from 1.38 g (W-R-Q) to 2.19 g (W-R-A-B-T) (Table 4). Free lipid (FL) was the most prominent fraction in terms of absolute content (Table 4) and as a percentage of total lipids (Fig. 1) in flour and dough samples; whereas protein-bound lipids (PBL) predominated in breads. Starchy lipids (SL) were minor lipid fraction in all single and blended matrices. FL in single grain flours varied greatly (from 0.75—rye—to 5.18—amaranth—, g/100 g flour basis, accounting from 45 to 89% of total lipids), little in blended flours (from 0.91—W-R—to 1.38—W-R-Q-A-B-T—, g/100 g flour basis), doughs (from 0.50—WRQ—to 0.83—WRA—, g/100 g flour basis, accounting from 14 to 47% of total lipids) and breads (from 0.42—WRBT—to 0.63—WRA—, g/100 g flour basis, accounting from 22 to 39% of total lipids). Individual addition of any grain flours significantly increased FL content, especially for quinoa; but in dough blended matrices, only amaranth presence promoted FL content (Table 3). SL was the intermediate lipid fraction in single flours (from 0.37—amaranth—to 0.59—quinoa—, g/100 g flour basis, accounting from 6 to 24% of total lipids), and blended flours (around 0.47 g/100 g flour basis), and doughs (from 0.39—WRQ—to 0.52—WRQAB—, g/100 g flour basis, accounting from 23— W-R-Q-A-B-T —to 39%—WRB—of total lipids). Finally, BL was the minor lipid fraction in single flours (from 0.28—quinoa, teff—to 0.49—rye—, g/100 g flour basis, accounting from 4—amaranth—to 30%—rye—of total lipids), and mixed flours (around 0.37 g/100 g flour basis). PBL predominated in breads (from 0.7 –WRQAB- to 1.28 –WRABT-, g/100 g flour basis, accounting from 43 –WRAAB- to 64% -WRQABT- of total lipids (Figure 1). The presence of single quinoa, amaranth or teff slightly decreased the

amount of BL in mixed flours (Table 3). SBL was the minor lipid fraction in breads (from 0.07—WRQA—to 0.49—WRQBT—, g/100 g flour basis, accounting from 5— WRQA—to 25%—WRQBT—of total lipids). The total lipid content determined in this study is in general in line with results previously found by Collar et al. (1998, 2001) for wheat, Schloenlechner et al., (2008) for pseudocereals, Lampi et al. (2004) for rye, and Hager et al. (2012) for pseudocereals and teff.

Relationships between dough and bread functional and nutritional properties and lipid binding during mixing and baking

Bread is a complex viscoelastic porous matrix, composed mainly of proteins/gluten, starch, lipids and water, whose sensory, technological and nutritional final quality is multifactor dependent. During dough mixing, flour particles are hydrated and sheared, and air incorporation takes place. At optimal mixing, in wheat flour based systems gluten proteins form a continuous network in which the starch granules and lipid components are dispersed. The binding of the initially free polar lipids confers a functional role on them in bread making. 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, interpherences in the binding of lipids to main biopolymers -protein, starch- can occur since original wheat flour system is diluted with other protein, starch and dietary fibre entities (Table 1) that compete for water and active sites of biomolecules. In this work, mixing induced binding of FL from flour to dough through a sharp decrease from -25% (W-R-A) to -82% (W-R-B) in the pool of free lipids with a concomitant increase in BL of 73% (W-R-A), 50% (W-R-B) and 76% (W-R-A-B) and a slight quantitative change in SL of +8%, +4% and -17%, respectively of blended doughs (Table 4). So that, a preferential lipid binding to the gluten/non gluten proteins and to the outside part of the starch granules takes place during mixing as previously observed for wheat (Collar et al., 1998) and non wheat matrices (Angolan and

Collar, 2011c). A slight displacement and/or accumulation of lipids in the SL fraction can also take place in accordance with the quantitative dynamics of lipids in the inside part of the starch granules (-17% - W-R-A-B- to +12% - W-T-Q-A-B-). Dynamics on FL and BL during mixing significantly ($p<0.01$) correlated (coefficient of correlation, r) with some functional and nutritional features in blended doughs and breads: the higher the decrease of FL content over mixing, the lower the pasting temperature ($r=0.801$), the holding strength ($r=0.8773$) and the viscosity of the hot paste ($r=0.8405$); the higher the accumulation of BL over mixing, the higher the bioaccessible polyphenol content ($r=0.8478$) in blended breads.

Baking transforms dough into a cellular product with a characteristic final texture and desirable eating properties. Major changes during baking are further expansion of the dough, drying of the surface (crust formation), and crust browning (Delcour and Hoseney, 2010). As the bread bakes from the outside to the inside, the crumb is baked. The starch granules swell and gelatinise, but their granular identity is largely retained at the water levels present in dough (Delcour and Hoseney, 2010). Whether amylose leaches out and to what extent (Goesaert et al., 2005) is unclear (Gray and Bemiller, 2003; Delcour et al., 2010).The transient gluten network formed in dough is transformed into a continuous, permanent network due to cross-linking at elevated temperatures (Goesaert et al., 2009b). During baking, gas cell opening occurs so that the bread is not only gluten continuous but also gas-continuous (Delcour and Hoseney, 2010). In this work, baking induced binding of FL and SL from dough to bread through a sharp decrease from -1% (W-R) to -39% (W-R-Q-A-T, W-R-A-B-T) in the pool of FL and from -12% (W-R-B) to -68% (W-R-Q-A-B-T) in the pool of SL, with a concomitant increase in PBL from 16% (W-R-Q), to 233% (W-R-A-B-T) of mixed breads (Table 4). This means that a preferential lipid binding to the gluten/non gluten proteins takes place during baking at the expenses of both a FL displacement and a lipid translocation/migration from the inside part of the starch granules to the protein 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). Dynamics on FL and SL during baking significantly ($p<0.01$) correlated with some functional features in blended doughs and breads: the higher the decrease of FL content over baking, the higher the pasting temperature ($r=0.8813$), and the lower the total setback on cooling ($r=0.8824$) and the dynamic moduli, but the higher the specific volume ($r=0.8756$) in blended breads. Complexation of lipids with amylose leached outside the granule can result in formation of an insoluble film at the granule surface that prevents transport of water inside the granule and, thus, further amylose leaching and granule swelling, decreases disruption of the granules and increases the gelatinisation temperature (Delcour et al., 2010). Dynamic moduli G' and G'' inform on the three-dimensional arrangement of dough. The lipid–starch interactions suggest a shift in the relaxation time of the dough cross-links to shorter times leading to a G' and G'' decrease and, consequently to a decrease in rigidity.

Conclusions

Dilution up to 20% of the basic rye/wheat flour blend by accumulative addition of amaranth, buckwheat, quinoa and teff flours (5% single flour) did impact ($p<0.01$) either some dough visco-metric and visco-elastic features, or in some techno-functional, nutritional and keeping characteristics of mixed bread matrices, and induced dynamics in lipid binding over mixing and baking steps, in variable extent.

Single addition of any of the non-wheat flours significantly promoted dietary fibre content, both soluble and insoluble fractions in breads, enhanced the gelling viscosity profile and the dough structure (increased dynamic moduli G', G'' values). Additionally, a slight delay in the pasting temperature and a decrease in the breakdown on cooking (quinoa), changes in the bioaccessible polyphenol content (decrease: quinoa; increase: amaranth). and a significant increase in the

antiradical activity (buckwheat) were achieved. Single amaranth and/ or teff provided slightly lower volume breads. Along breadmaking, a preferential lipid binding to the gluten/non gluten proteins and to the outside part of the starch granules takes place during mixing, in such a way that the higher the accumulation of bound lipids over mixing, the higher the bioaccessible polyphenol content in blended breads. During baking, again

a preferential lipid binding to the gluten/non gluten proteins takes place during baking at the expenses of both a free lipid displacement and a lipid translocation/migration from the inside part of the starch granules to the protein active sites. Dynamics on free and starchy lipids during baking significantly correlated with some functional features in blended doughs and breads: the higher the decrease of free lipid content over baking, the higher the pasting temperature, and the lower the total setback on cooling and the dynamic moduli, but the higher the specific volume in blended breads.

Addition of amaranth, buckwheat, quinoa and teff flours at 20% to a wheat:rye (50:50, w:w) flour matrix allowed to obtain enhanced-value grain-based breads in terms of higher nutritional value and health-promoting impact, preserving the techno-functional performance and the sensory appreciation of breads thereof. Improvement is concomitant with the dynamics of lipid fractions during mixing and baking.

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Table 1.- Chemical, functional and nutritional composition of flours (mean of three replicates ± SD).

Parameter	Flours					
	Amaranth	Buckwheat	Rye	Teff	Quinoa	Wheat
Moisture, g/ 100 g flour, as is	12.31±0.29a	13.86±0.32c	12.64±0.32b	11.89±0.27a	12.05±0.36ab	14.32±0.32c
Fat, g/ 100 g flour, as is	5.08±0.18f	2.52±0.09c	0.93±0.10a	4.46±0.24e	3.44±0.19d	1.34±0.09b
(%, dry basis)	(5.79)	(2.93)	(1.06)	(5.06)	(3.91)	(1.56)
Ash, g/ 100 g flour, as is	1.59±0.05c	1.67±0.09c	0.81±0.08b	1.95±0.09d	2.10±0.10d	0.54±0.06a
(%, dry basis)	(1.81)	(1.94)	(0.93)	(2.21)	(2.39)	(0.63)

Protein, g/ 100 g flour, as is (%, dry basis)	11.00±0.23b (12.54)	13.07±0.15e (15.17)	8.92±0.11a (10.21)	11.50±0.12c (13.05)	11.32±0.16bc (12.87)	12.11±0.21d (14.13)
Insoluble Fibre, g/ 100 g flour, as is (%, dry basis)	7.91±0.85cd (9.02)	5.81±0.75b (6.74)	7.81±0.95cd (8.94)	6.52±0.38bc (7.40)	9.13±0.96d (10.38)	1.28±0.09a (1.49)
Soluble Fibre, g/ 100 g flour, as is (%, dry basis)	5.66±0.95c (6.45)	6.12±1.02c (7.10)	4.42±0.96bc (5.06)	4.23±0.75b (4.80)	5.37±1.03bc (6.11)	0.91±0.09a (1.06)
Total Dietary Fibre, g/ 100 g flour, as is (%, dry basis)	13.57±1.46cd (15.47)	11.94±1.32bc (13.86)	12.23±1.09bc (14.00)	10.74±1.03b (12.19)	14.5±0.98d (16.49)	2.19±0.09a (2.56)
*Digestible carbohydrates, g/ 100 g flour, as is (%, dry basis)	56 (63.86)	56 (65)	64 (73.26)	59 (67)	57 (65)	70 (82)
Water Holding Capacity 3000 rpm, g water/g flour	1.44±0.10c	1.13±0.09b	1.16±0.12b	1.36±0.11bc	1.57±0.09c	0.84±0.06a
Water Holding Capacity 5000 rpm, g water/g flour	1.05±0.05c	0.8±0.09ab	0.91±0.07b	1±0.09bc	1.16±0.06c	0.61±0.09a
Fat Adsorption Capacity, g/g	1.54±0.12bc	1.33±0.09a	1.71±0.13c	1.35±0.11ab	1.2±0.10a	1.38±0.12ab
Foam Capacity, mL	9±2b	3±1a	26±2d	5±1a	3±1a	14±2c
Foam Stability 30', %	44±3b	100±6d	96±9d	60±5c	100±7d	36±2a
Foam Stability 60', %	33±3a	100±5d	81±8c	60±4b	100±4d	36±3a
Solvent Retention Capacity, %						
Water	84±5b	84±7b	105±8c	111±6c	134±8d	61±4a
Sucrose	106±5b	114±7b	157±8cd	145±8c	166±9d	91±6a
Sodium carbonate	99±4b	84±8a	146±7d	120±9c	131±8cd	81±6a
Lactic acid	98±4a	93±6a	110±6b	129±8cd	140±8d	111±9b

*Calculated by difference.

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

Table 2.- Visco-metric and visco-elastic parameters of multigrain doughs and techno-functional and nutritional features of fresh and stored breads thereof (mean values ± standard deviation).

	Property/parameter	Sample*															
		WR	WRQ	WRA	WRB	WRT	WRQA	WRQB	WRQT	WRAB	WRAT	WRBT	WRQAB	WRQAT	WRABT	WRQBT	WRQABT
Dough	*Viscometric*																
	Peak Viscosity, cP	1631±24	1646±30	1629±23	1692±32	1608±20	1622±30	1627±34	1651±18	1572±40	1701±25	1597±10	1574±15	1606±20	1576±18	1633±10	1574±10
	Pasting Temperature, ºC	86.7±0.3	87.6±0.2	85.8±0.4	88.00±0.4	87.4±0.3	88.0±0.2	87.1±0.4	88,00±0.4	88,00±0.4	87.5±0.2	86.6±0.1	88.3±0.1	88.4±0.3	86.5±0.1	87.8±0.2	87.9±0.1
	Holding strength, cP	862±10	868±14	873±12	923±20	886±11	906±15	877±18	903±14	882±12	885±8	874±10	868±9	875±10	871±11	930±11	887±8
	Breakdown, cP	769±10	778±17	757±6	769±8	722±12	716±16	750±18	748±10	690±13	817±9	723±8	706±8	731±12	705±6	703±7	688±7
	Viscosity at 95ºC, cP	259±13	238±12	257±10	261±10	269±8	257±6	269±11	253±11	263±10	274±10	248±9	243±3	228±9	246±9	226±10	245±6
	Viscosity at end of 95ºC, cP	976±20	1043±33	1030±12	1142±21	989±32	1040±15	1040±16	1057±14	1063±21	1075±13	1048±10	1035±21	1041±18	1039±14	1163±14	1064±16
	Viscosity at 50ºC, cP	1384±40	1384±34	1388±41	1366±24	1405±14	1452±30	1393±8	1414±40	1348±40	1437±40	1368±40	1389±18	1425±21	1420±11	1404±18	1444±13
	Final Viscosity, cP	1794±8	1821±10	1833±21	1846±16	1821±14	1943±23	1856±15	1876±20	1831±36	1955±12	1854±13	1911±18	1968±20	1965±31	1926±16	1995±14
	Total setback, cP	932±12	953±6	961±11	923±16	935±15	1038±6	979±9	973±8	949±7	1070±11	981±10	1043±12	1093±9	1094±7	997±7	1108±13
	Visco-elastic																
	Storage modulus, Pa	4004±95	3647±65	3496±46	2982±74	3960±85	4449±73	4263±79	4765±89	3897±72	4221±86	4166±74	4687±61	5054±27	4420±85	4371±87	6010±49
	Loss modulus, Pa	2384±63	2235±96	2119±38	1837±26	2359±64	2605±79	2616±62	2911±38	2361±47	2498±39	2551±72	2871±47	3056±83	2699±95	2667±99	3638±34
Fresh bread	*Techno-functional*																
	Overall acceptability (/10)	6±1	7±1	7±1	7±1	7±1	6±1	6±1	6±1	5±1	5±1	5±1	4±1	4±1	5±1	5±1	5±1
	Specific volume, mL/g	3.1±0.2	3.0±0.3	3.1±0.1	3.2±0.1	3.0±0.2	2.8±0.1	2.9±0.1	2.78±0.2	2.82±0.1	2.52±0.3	2.67±0.3	2.47±0.3	2.56±0.2	2.64±0.2	2.74±0.2	2.70±0.2
	Aspect ratio	1.6±0.2	1.7±0.2	1.8±0.3	1.6±0.2	1.6±0.2	1.6±0.1	1.5±0.1	1.8±0.1	1.8±0.2	1.5±0.1	1.7±0.1	1.7±0.2	1.6±0.2	1.6±0.2	1.6±0.1	1.7±0.3
	Cohesiveness	0.79±.03	0.75±.01	0.75±.04	0.73±.06	0.73±.04	0.74±.01	0.74±.03	0.77±.01	0.75±.02	0.74±.02	0.70±0.1	0.71±.06	0.76±.05	0.75±.04	0.73±.03	0.73±.02
	Springiness	0.99±.03	0.99±.06	0.96±.07	0.97±.06	0.97±.01	0.96±.08	0.96±.04	0.98±.04	0.96±.02	0.97±.02	0.96±.02	0.94±.05	0.94±.01	0.96±.02	0.98±.02	0.94±.02
	Resilience	.43±.003	.39±.001	.38±.003	.37±.002	.38±.006	.37±.004	0.38±.006	0.4±.008	0.39±.008	.38±.003	.33±.001	.35±.003	.39±.002	.37±.002	.37±.006	.37±.002
	L	57±3	59±2	59±5	58±4	61±2	60±1	60±1	59±2	60±3	61±6	62±5	59±2	60±4	62±7	58±3	57±3
	a	1.58±.09	1.79±.06	1.62±.06	2.15±.07	1.92±.05	1.83±.02	2.07±.01	1.9±.01	1.85±.01	1.97±.02	1.92±.01	2.13±.01	2,00±.03	2.07±.05	2.2±.06	2.32±.06

	b	14.77±.11	15.59±.15	15.01±.11	15.64±.13	16.2±.15	16.01±.16	15.86±.21	15.94±.11	15.58±.11	16.69±.13	16.11±.11	16.1±.10	16.7±.12	16.54±.12	16.03±.11	15.81±.10
	Nutritional, % bread as is															
	Fat,	2.93±.09	2.91±.12	2.93±.10	2.93±.14	3.00±.20	3.03±.16	2.93±.09	2.92±.08	2.99±.10	2.99±.12	2.95±.08	2.95±.08	3.01±.09	2.94±.13	3.01±.14	3.09±.12
	Protein	8.28±.24	8.27±.36	8.20±.24	8.46±.19	8.46±.31	8.52±.26	8.48±.19	8.27±.27	8.53±.36	8.32±.19	8.47±.26	8.43±.21	8.45±.18	8.49±.12	8.55±.09	8.80±.16
	IDF	2.59±.38	2.74±.49	2.69±.53	2.70±.37	2.73±.48	2.94±.57	2.85±.43	2.81±.59	2.84±.35	2.80±.43	2.78±.39	2.95±.58	2.98±.34	2.92±.32	2.92±.46	3.14±.51
	SDF	1.52±.36	1.61±.44	1.60±.32	1.66±.57	1.61±.64	1.75±.59	1.75±.34	1.66±.32	1.77±.41	1.68±.42	1.71±.37	1.82±.33	1.79±.51	1.80±.18	1.82±.09	1.95±.15
	DC	44	44	45	44	45	44	44	44	45	44	45	44	45	44	43	43
	Free phenolics, mg	287±23	308±18	290±34	350±41	303±23	343±27	352±33	314±37	365±44	284±39	342±46	273±53	342±24	311±19	283±31	256±25
	Flavonoids, mg	334±62	301±47	340±31	332±38	347±42	341±66	334±21	327±23	425±43	348±61	310±37	264±31	357±44	379±29	390±33	344±25
	Anti-radical activity, %	39±3	38±6	41±4	43±4	41±2	41±7	47±5	47±6	50±3	36±8	46±7	44±7	47±2	48±2	51±5	43±4
	Bioaccessible polyphenols, mg	1021±89	1010±73	1134±58	1007±44	1036±71	1042±29	1008±66	942±48	1070±38	1147±75	1119±81	971±96	1109±44	1090±37	999±41	1067±96
Stored bread	*Staling kinetics*																
	$T_{0,}$ N	5.71±.21	5.07±.32	6.6±.26	6.09±.84	6.37±.33	6.41±.24	7.02±.51	5.62±.59	6.24±.39	6.31±.74	6.43±.68	7.2±.73	6.48±.84	7.21±.67	5.96±.34	9.42±.69
	$T_{\infty,}$ N	12.1±.92	19.91±.87	23.12±.99	24.17±.71	14.18±.49	54.02±.88	16.08±.96	20.39±.99	15.92±.47	39.24±78	55.5±96	59.2±32	46.26±66	40.04±74	29.94±95	32.83±87
	n	0.61±.06	0.65±.08	0.57±.09	0.59±.12	1.48±.32	0.81±.08	0.56±.08	1.05±0.6	0.72±.07	0.73±.05	0.49±.09	0.56±.04	0.27±.05	0.52±.07	0.59±.07	0.46±.03
	k	0.36±.03	0.26±.06	0.18±.06	0.11±.09	0.17±.04	0.04±.00	0.4±.01	0.12±.03	0.43±.12	0.08±.03	0.09±.06	0.08±.02	0.14±.05	0.13±.03	0.15±.08	0.16±.01

(*) Blended samples were based on: wheat (W), rye (R), quinoa (Q), amaranth (A), buckwheat (B), and teff (T).

Table 4.-Lipid fractions (g/100 g of flour) from single flours and blended flours, doughs and breads thereof (mean values of three replicates± standard deviation).

Samples	Free lipids			Bound lipids		Protein-bound	Starchy Lipids		Starch-bound	Total Lipids		
	Flour	Dough	Bread	Flour	Dough	Bread	Flour	Dough	Bread	Flour	Dough	Bread
W-R	0.91±0.09	0.53±0.09	0.52±0.06	0.39±0.10	0.49±0.05	1.11±0.10	0.47±0.09	0.42±0.07	0.27±0.10	1.76	1.43	1,90
W-R-Q	1.02±0.07	0.50±0.08	0.45±0.08	0.38±0.12	0.54±0.09	0.63±0.09	0.47±0.08	0.39±0.09	0.30±0.08	1.87	1.44	1,38
W-R-A	1.11±0.10	0.83±0.10	0.63±0.11	0.38±0.09	0.65±0.07	0.98±0.13	0.46±0.06	0.50±0.12	0.35±0.07	1.95	1.98	1,96
W-R-B	0.98±0.12	0.18±0.02	0.56±0.12	0.38±0.13	0.57±0.07	1.17±0.16	0.47±0.12	0.49±0.14	0.43±0.12	1.83	1.24	2,15
W-R-T	1.06±0.09	0.55±0.08	0.54±0.06	0.38±0.08	0.53±0.11	1.03±0.24	0.47±0.15	0.50±0.09	0.13±0.09	1.91	1.57	1,69
W-R-Q-A	1.21±0.11	0.61±0.10	0.57±0.11	0.37±0.09	0.49±0.12	0.83±0.13	0.47±0.18	0.39±0.10	0.07±0.02	2.05	1.49	1,47
W-R-Q-B	1.08±0.13	0.62±0.09	0.47±0.10	0.38±0.12	0.57±0.05	1.22±0.15	0.47±0.09	0.47±0.21	0.29±0.12	1.93	1.66	1,98
W-R-Q-T	1.16±0.15	0.64±0.09	0.53±0.07	0.38±0.15	0.48±0.16	0.88±0.09	0.47±0.12	0.56±0.18	0.24±0.11	2.01	1.68	1,65
W-R-A-B	1.17±0.11	0.53±0.12	0.56±0.09	0.38±0.20	0.66±0.17	0.82±0.06	0.46±0.15	0.39±0.16	0.19±0.09	2.01	1.58	1,56
W-R-A-T	1.25±0.15	0.75±0.08	0.47±0.13	0.37±0.08	0.60±0.09	0.88±0.08	0.46±0.08	0.50±0.19	0.17±0.06	2.08	1.85	1,52
W-R-B-T	1.13±0.16	0.62±0.13	0.42±0.12	0.38±0.13	0.55±0.11	1.08±0.09	0.47±0.13	0.47±0.13	0.29±0.08	1.97	1.63	1,78
W-R-Q-A-B	1.26±0.12	0.64±0.08	0.52±0.09	0.37±0.08	0.50±0.06	0.61±0.13	0.47±0.23	0.52±0.16	0.30±0.14	2.10	1.66	1,43
W-R-Q-A-T	1.33±0.09	0.76±0.11	0.46±0.10	0.37±0.06	0.56±0.09	1.11±0.18	0.47±0.21	0.46±0.15	0.29±0.14	2.17	1.78	1,86
W-R-A-B-T	1.22±0.11	0.79±0.13	0.48±0.12	0.37±0.07	0.39±0.06	1.28±0.16	0.47±0.19	0.50±0.18	0.43±0.13	2.06	1.68	2,19
W-R-Q-B-T	1.30±0.09	0.65±0.21	0.46±0.09	0.37±0.09	0.71±0.09	1.03±0.08	0.46±0.09	0.40±0.14	0.49±0.16	2.14	1.76	1,98
W-R-Q-A-B-T	1.38±0.12	0.71±0.11	0.49±0.06	0.37±0.09	0.43±0.05	1.14±0.15	0.47±0.15	0.44±0.16	0.14±0.14	2.22	1.58	1,77
Wheat	1.06a			0.28a			0.51a			1.85a		
Rye	0.75a			0.49b			0.42a			1.66a		
Quinua	3.23b			0.28a			0.59a			4.09c		
Amaranth	5.18d			0.26a			0.37a			5.81d		
Buckwheat	2.50b			0.32a			0.54a			3.36b		
Teff	4.14c			0.28a			0.48a			4.91c		

Within each column of single flour samples, values with different letters differ significantly from each other ($p < 0.05$). Blended samples were based on: wheat (W), rye (R), quinoa (Q), amaranth (A), buckwheat (B), and teff (T).

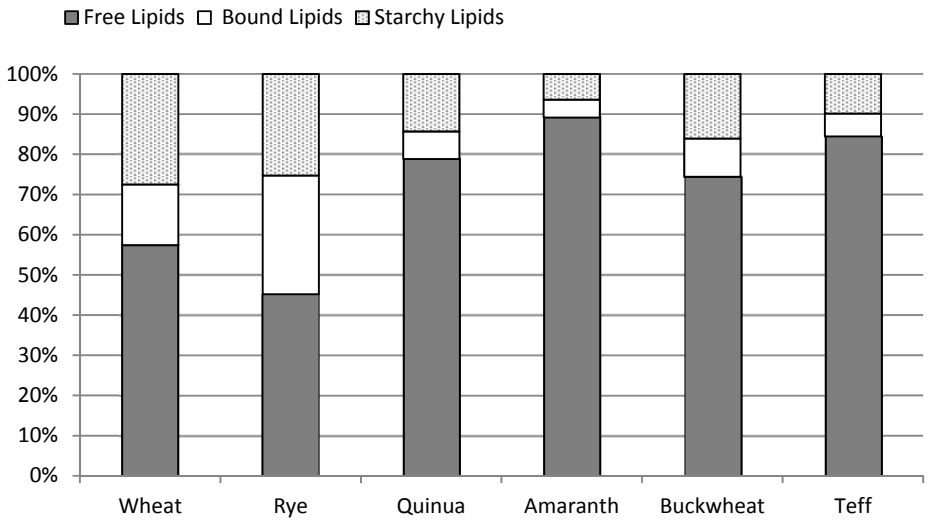
Table 3.- Significant (p<0,01) single effects of quinua, amaranth, buckwheat and teff on the lipid and functional profiles along breadmaking of mixed grain matrices.

Parameter	Overall mean	Level	Design factor							
			Quinoa		Amaranth		Buckwheat		Teff	
Free Lipids flour, g/100 g flour as is	1.161	1	1.104	a	1.080	a	1.131	a	1.093	a
		2	2.218	b	1.241	b	1.190	b	1.229	b
Free Lipids dough, g/100 g flour as is,	0.619	1	ns		0.536	a	ns		ns	
		2			0.703	b				
Bound Lipids flour, g/100 g flour as is	0.376	1	0.379	b	0.380	b	ns		0.379	b
		2	0.374	a	0.373	a			0.374	a
Pasting Temperature, ºC	87.48	1	87.06	a	ns		ns		ns	
		2	87.89	b						
Breakdown, cP	736	1	755	b	ns		ns		ns	
		2	717	a						
Final viscosity, cP	1887	1	1862	a	1849	a	ns		1854	a
		2	1912	b	1925	b			1920	b
Total setback, cP	1002	1	981	a	959	a	ns		972	a
		2	1023	b	1045	b			1031	b
Storage modulus, Pa*	4275	1	3893	a	4020	a	ns		3928	a
		2	4656	b	4529	b			4621	b
Loss modulus, Pa*	2589	1	2351	a	ns		ns		2379	a
		2	2825	b					2797	b
Specific volume, mL/g	2.81	1	ns		2.93	b	ns		2.93	b
		2			2.70	a			2.70	a
Cohesiveness	0.74	1	ns		ns		0.751	b	ns	
		2					0.729	a		
Resilience	0.378	1	ns		ns		0.390	b	ns	
		2					0.366	a		
a	1.96	1	ns		ns		1.83	a	1.88	a
		2					2.09	b	2.04	b
b	15.91	1	ns		15.77	a	ns		15.57	a
		2			16.06	b			16.25	b
IDF	2.84	1	2.76	a	2.76	a	2.79	a	2.79	a
		2	2.92	b	2.91	b	2.89	b	2.89	b
SDF	1.72	1	1.67	a	1.67	a	1.65	a	1.69	a
		2	1.77	b	1.77	b	1.79	b	1.75	b
Antiradical activity, %	44	1	ns		ns		41	a	ns	

		2					47	b	
Bioaccessible polyphenols, mg/100 g bread, as is	1048	1	1078	b	1018	a	ns		ns
		2	1018	a	1079	b			
T∞	26.43	1	ns		24.03	a	ns		ns
		2			28.83	b			

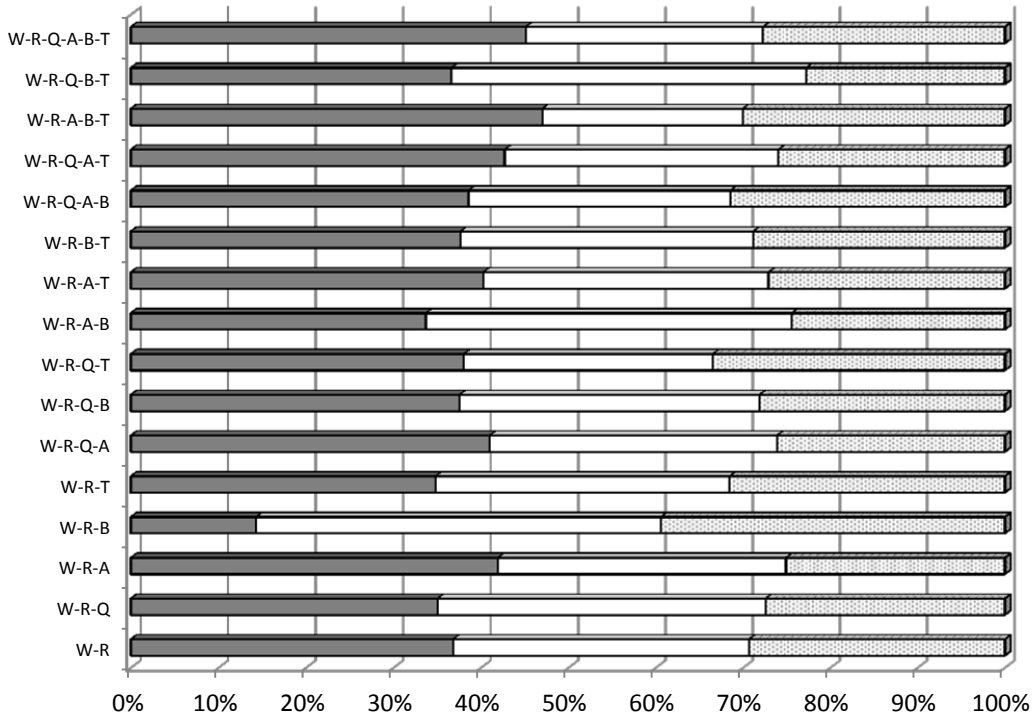
Levels: absence (1), presence (2). Values with different letters differ significantly from each other ($p < 0.05$).

(*) Dynamic moduli values were taken at a frecuency of 1 Hz.


Free Lipids
Bound Lipids
Starchy Lipids
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Wheat
Rye
Quinua
Amaranth
Buckwheat
Teff

(A)

18


W-R-Q-A-B-T
W-R-Q-B-T
W-R-A-B-T
W-R-Q-A-T
W-R-Q-A-B
W-R-B-T
W-R-A-T
W-R-A-B
W-R-Q-T
W-R-Q-B
W-R-Q-A
W-R-T
W-R-B
W-R-A
W-R-Q
W-R
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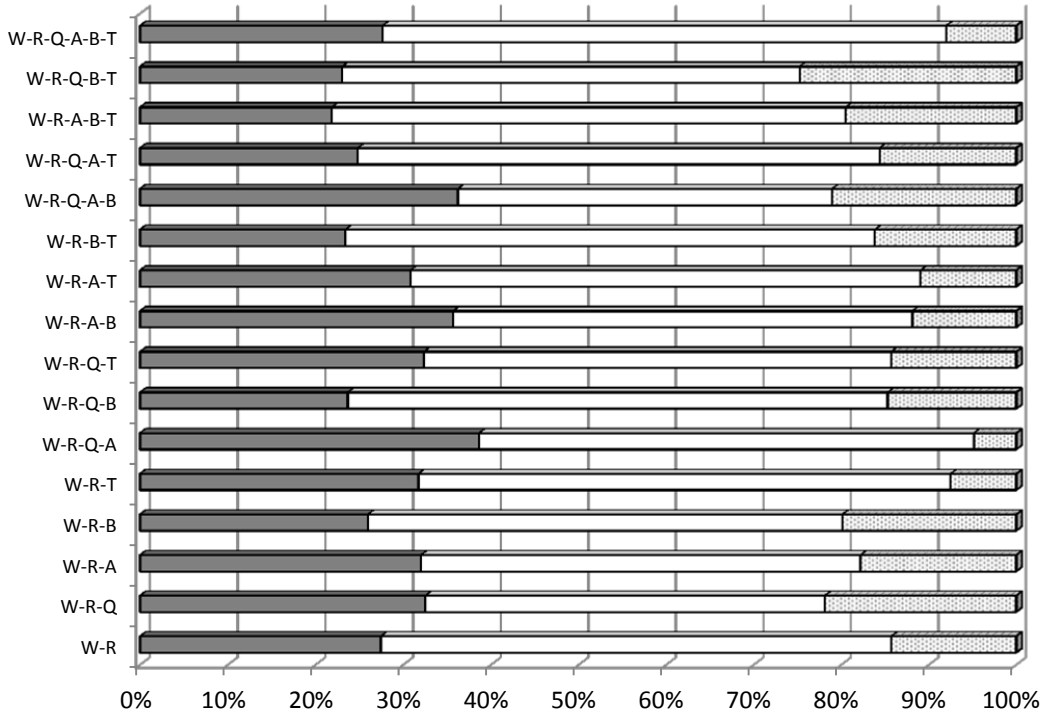
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

(B)

19



(C)

Figure 1.- Lipid distribution (%) in flour (A), dough (B) and bread (C) samples. Blended samples were based on: wheat (W), rye (R), quinoa (Q), amaranth (A), buckwheat (B), and teff (T).