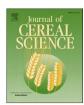
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Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs



Effect of bran on bread staling: Physico-chemical characterization and molecular mobility



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ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form
16 March 2015
Accepted 10 June 2015
Available online 18 June 2015

Keywords: Wheat bran Bread ¹H NMR molecular mobility Staling

ABSTRACT

High fibre breads were produced with the addition of durum wheat bran fractions (regular bran and a fraction extracted from the most internal bran layer) and their physico-chemical properties and water status were characterised during storage. Bran enriched breads exhibited similar properties during storage, they were harder, less springy and less cohesive than the control. Water status was strongly affected by bran addition, independently of bran composition: water activity, moisture and frozen water content (measured by Differential Scanning Calorimetry) were generally higher in the bran samples than in the control bread during storage. Amylopectin retrogradation was significantly larger in the presence of bran fractions. ¹H NMR mobility (T₂ number of populations and relaxation times) was different in the high fibre breads as compared to the control sample. The changes in protons mobility observed upon storage indicated an influence of bran on water/gluten/starch molecular domains and their dynamics, that may have affected the development of the gluten network resulting in different textural properties.

1. Introduction

Bread is an important staple food for western populations, and fibre enriched products might be a good way to increase the fibre intake in these individuals. Dietary fibre is known to have multiple beneficial effects on human health (Redgwell and Fischer, 2005) and an increase in its intake should be encouraged. According to the European legislation (EC Regulation n.1924/2006), a bread enriched with fibre can be defined as a high fibre product (and therefore able to promote beneficial physiological effects when consumed) if contains at least 6 g of fibre in 100 g of product. Addition of fibre to a bread formulation significantly modifies dough and final product properties, alters the production process (Collar et al., 2007), and affects staling-related phenomena (e.g. gluten dehydration, amorphous starch recrystallization, water molecular redistribution among bread components; Fadda et al., 2014; Gray and Bemiller, 2003).

Effects of bran addition on dough and bread properties have been extensively investigated. Many studies have focused on the

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action of pentosans (that represents ~30% of the bran) on bread quality (Wang, et al., 2003a,b; Wang et al., 2004a,b) and staling (Fessas and Schiraldi, 1998). Water-extractable pentosans have been reported to have a positive effect on loaf volume and starch retrogradation in bread (Kim and D'Appolonia, 1977). A negative action of the water un-extractable fraction of pentosans was reported in terms of decreased gas retention (Courtin and Delcour, 2002), dilution (Gan et al., 1992) and physical hindrance (Lai et al., 1989) of gluten network. More recently Noort et al. (2010) hypothesised the establishment of arabinoxylans gluten interactions that are probably responsible for an altered structure in bran enriched breads. Curti et al. (2013) investigated the effect of bran on fresh bread properties and reported that bran altered product water status at all structural levels (from molecular to macroscopic), reduced loaf volume and increased crumb hardness (at a different degree depending on bran composition).

Water status is known to have an important effect on bread staling (Gray and Bemiller, 2003), and, therefore, the effect of the addition of wheat bran fractions (with different composition) on bread staling has been investigated with a focus on the state of water and molecular mobility measured by time domain ¹H NMR relaxometry.

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2. Experimental

2.1. Bread formulation, production and storage

Bread loaves were produced with the formulations [wheat flour (Molino Seragni, Cremona, Italy); sugar (Coprob S.C.A, Pavia, Italy); salt (Italkali s.p.a., Palermo, Italy); yeast (AB Mauri Italy s.p.a, Padova, Italy); sunflower seeds oil (Oleificio Zucchi, Cremona, Italy)] reported in Table 1. Wheat flour [water 14%, gluten 11% dry matter (dm), fibre 2.5%dm, starch 84.55%dm, ashes 0.65%] was used for the production of the control sample, that was named STD. Wheat flour was partially replaced with two wheat bran fractions to obtain a high fibre product (as required by the EU regulation 1924/2006) with a total fibre content of 6.5% (g fibre/100 g bread). Bran was taken either as it (BRA) or extracted from the most internal bran layer (INT), and milled to a particle size <500 μ m. Bran compositions (AOAC Official Methods, 1995, 2003) were:

- BRA [water 8.1 (%), proteins 13.7 (%dm), fat 5.3 (%dm), total dietary fibre 52 (%dm), starch 28.5 (%dm), ashes 5.8 (%dm)].
- INT [water 12.0 (%), proteins 18.6 (%dm), fat 6.3 (%dm), total dietary fibre 36 (%dm), starch 38.9 (%dm), ashes 6.5 (%dm)].

The water amount to be added to each bread formulation was taken by the measurement of farinograph water absorption to 500 LIR

Breads were produced with a home bread-maker (Severin BM3986, Germany) using a "wholemeal" program (first kneading 43 min; second kneading 56 min; first fermentation 45 min; smoothing 1 min; second fermentation 18 min; smoothing 1 min; third fermentation 35 min; baking 55 min), cooled to room temperature, placed in polyethylene bags sprinkled with about 3 ml ethanol, and stored at room temperature. Three loaves were produced for each sample at each storage time. Samples were analysed fresh (day 0) and after 1, 3, 5 and 7 days of storage.

2.2. Bread characterization

2.2.1. Crumb texture

Bread crumb hardness, springiness and cohesiveness were measured with a TA.TX2 Texture Analyzer (Stable Micro Systems, Goldalming, UK). At least eight cubic portions ($2 \times 2 \times 2$ cm) of crumb were extracted from the central slices of the bread loaf and compressed (trigger force = 0.1 N) to 40% deformation using a cylindrical probe (P/35 Dia Cylinder Aluminium). Hardness (height of the first compression peak), cohesiveness (ratio area second/first compression peaks) and springiness (ratio length second/first compression peaks) were determined.

2.2.2. Moisture content

Moisture content of crumb (from loaf centre) and crust were determined in triplicate for each bread loaf at each storage time by weight loss at 105 $^{\circ}$ C (NSV 9035, ISCO, Milan, Italy) to constant

Table 1Bread formulations.

Ingredient (%)	STD	INT	BRA
Wheat flour	100.0	76.5	84.0
Bran	_	23.5	16.0
Sugar	4.0	4.0	4.0
Salt	2.0	2.0	2.0
Yeast	3.0	3.0	3.0
Water	55.2	61.2	63.3
Sunflower seed oil	3.0	3.0	3.0

weight.

2.2.3. Frozen water content and retrograded amylopectin

Thermal properties of bread crumb were measured using a Differential Scanning Calorimeter (DSC Q100 TA Instruments, New Castle, DE, USA), calibrated with indium and mercury. Bread crumb (4 g, from loaf centre) was compressed with a 2.5 kg weight to obtain a flat and compact crumb sample to maximize heat transfer within the DSC cell during the experiment. Compressed crumb samples (5–10 mg) were taken and placed in stainless steel pans (Perkin Elmer, USA) that were then hermetically sealed, quench cooled to $-80~^{\circ}\text{C}$ and then heated to 130 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$. DSC thermograms were analysed (Universal Analysis Software 3.9A, TA Instruments, New Castle, DE) and "frozen" water content (at the given experimental conditions; FW) was calculated from the endothermic peak around 0 $^{\circ}\text{C}$ (ice melting) using the following equation:

$$FW = Enthalpy \ lce \ Fusion \times \left(\frac{1}{\textit{latent heat ice fusion}}\right) \times \left(\frac{1}{\textit{MC}}\right) \\ \times 100$$

Where FW is Frozen water at the given experimental conditions (g frozen water/100 g water), Enthalpy Ice Fusion (J/g product), Latent heat of ice fusion is 334 J/g ice and MC is Moisture Content (g water/g product).

Retrograded amylopectin (J/g of dry starch) was obtained by the integration of the endothermic peak in the $50-80\,^{\circ}\text{C}$ temperature range, expressed on the starch content (dry basis) of each sample.

2.2.4. Molecular mobility (¹H NMR)

A low resolution (20 MHz) ¹H NMR spectrometer (the MiniSpec. Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1 °C was used to study a range of proton molecular mobility by measuring the Free Induction Decay (FID) and transverse (T₂) relaxation time. Three g of compressed bread crumb (15 mm high, extracted from loaf centre) were placed into a 10 mm NMR tube that was then sealed with Parafilm® to prevent moisture loss during the NMR experiment. FIDs were acquired using a single 90° pulse, followed by dead time of 7 µs and a recycle delay of 3 s. T₂ (transverse relaxation times) curves were obtained with a CPMG pulse sequence with a recycle delay of 3 s, 32 scans (to increase the signal-to-noise ratio), an interpulse spacing of 0.04 ms and preselected data points (3000-4000) depending on sample. T2 curves were analysed as quasi-continuous distributions of relaxation times using a UPEN-Win software. Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to avoid extrapolation of relaxation times shorter than the first experimental point.

2.3. Statistical analysis

One-way-analysis of variance (ANOVA, SPSS v.16, SPSS Inc. IL, USA) was used to verify significant differences ($p \le 0.05$) of evaluated parameters of the same sample during storage and among the samples at the same storage time.

3. Results and discussion

3.1. Crumb texture

Crumb hardness of STD, INT and BRA samples during storage is shown in Table 2. Bran addition in bread formulation altered textural properties of fresh and stored breads. INT was significantly

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