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Oxidative stability of whole wheat bread during storage

Sidsel Jensen [a,c], Henrik Oestdal [b], Morten R. Clausen [c], Mogens L. Andersen [a], Leif H. Skibsted [a,*]

[a] Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
[b] Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark
[c] Department of Food Science, Faculty of Agricultural Sciences, Aarhus University, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

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ABSTRACT

The oxidative stability was examined in whole wheat bread packed in modified atmosphere (nitrogen) using vacuum grade plastic bags and stored for up to 5 weeks. Small, but significant, differences in oxidative stability developed with time for whole wheat bread crumb and crust. The samples were evaluated by direct electron spin resonance (ESR) spectroscopy for detection of free radicals, peroxide value (POV), overall antioxidative capacity using Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays, and by the content of tocopherols as determined by HPLC. The overall antioxidative capacity was reduced during storage with an accumulation of lipid hydroperoxides peaking after 2–3 weeks of storage. Bread crust was generally found to be more oxidative stable when compared to crumb. Quality of bread with extended shelf life may accordingly be improved minimising oxidation.

1. Introduction

Bread producers are highly focused on controlling the rate of bread firming and microbial growth allowing bread with a shelf life of up to 4 weeks to be produced (Sargent, 2008). Control of bread firming and microbial activity mean that other quality parameters may determine the shelf life of baked products. It has previously been shown that changes in aroma, flavour and taste occur during storage of whole wheat bread (Holtekjoelen, Baevre, Roedbotten, Berg, & Knutsen, 2008; Jensen, Oestdal, & Thybo, 2010). In these studies aroma and flavour attributes such as 'acidic', 'off', 'rancid' and 'dust' together with the taste attributes 'sweet' and 'bitter' were found to increase with storage time. Similar attributes have previously been shown to correlate with oxidation of food products in general (Angerosa, 2002; Paradiso, Summo, Pasqualone, & Caponio, 2009). However, a thorough study of the oxidative stability of bread during extended storage has, to the best of our knowledge, not been reported. Characterisation of the early oxidative changes occurring during storage should be valuable for the understanding of quality changes for such products and may even provide a tool for shelf life prediction of bread products. Industrialisation of the bread production necessitates development of products with a high degree of product stability in order to provide the required extended shelf life (Sewald & DeVries, 2009).

The stability of whole wheat flour and bread is affected by the lipid composition and the presence of different antioxidants. The lipid content of whole wheat flour is in the range of 1.3–2.0% depending on wheat type and milling method and the main fatty acid is the unsaturated linoleic acid ($C_{18:2}$), which constitutes 52–57% (w/w) of the fatty acids (Prabhasankar & Rao, 2001). The present whole wheat bread was prepared with the addition of soybean oil which especially increases the content of unsaturated linoleic acid in these bread loaves. Oxidation of unsaturated fatty acids produces a complex mixture of volatiles that significantly affects sensory properties of foods even when present in low quantities. Tocopherols (lipid soluble) and phenolic acids (water soluble) are responsible for the largest part of the antioxidative activity in wheat (Zhou, Yin, & Yu, 2005), and are known to vary in content between wheat variety and growing conditions (Yu, Haley, Perret, & Harris, 2002). Furthermore, compounds formed during baking as a consequence of the Maillard reactions are known to possess antioxidative activity and such compounds could impact overall oxidative stability of baked products positively (Lindenmeier, Faist, & Hofmann, 2002; Michalska, Amigo-Benavent, Zielinski, & del Catillo, 2008).

Accordingly, it is of interest to monitor oxidative changes in bread from the initial formation of free radicals. The aim of the

Abbreviations: ANOVA, analysis of variance; ESR, electron spin resonance; HPLC, high performance liquid chromatography; ORAC, oxygen radical absorbance capacity; POV, peroxide value; SD, standard deviation; TEAC, trolox equivalent antioxidant capacity.

* Corresponding author. Tel.: +45 35333221.
E-mail addresses: sije@agrsci.dk (S. Jensen), hnqs@novozymes.com (H. Oestdal), mortenr.clausen@agrsci.dk (M.R. Clausen), mola@life.ku.dk (M.L. Andersen), ls@life.ku.dk (L.H. Skibsted).

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present study was to measure oxidative changes of both crust and crumb of whole wheat bread stored for up to 5 weeks using modified atmosphere packaging (MAP). MAP of bread does not ensure oxygen free conditions due to limitations of how much oxygen that can be removed from inside baked products (Piergiovanni & Fava, 1997) and oxygen transmission through plastic films. Oxidative changes were followed by the use of ESR spectroscopy, analysis of lipid hydroperoxides by determination of POV, determination of overall antioxidative capacity by the TEAC assay and the ORAC assay, and by measurements of the tocopherol content. ESR spectroscopy has on different food products with low or intermediate water activity proven effective in shelf life prediction (Jensen, Danielsen, Bertelsen, Skibsted, & Andersen, 2005; Nissen, Mansson, Bertelsen, Huynh-Ba, & Skibsted, 2000), but has never been used on bread.

2. Materials and methods

2.1. Bread samples

The experiment included whole wheat yeast-leavened bread prepared according to sponge and dough procedure. The sponge was made from parts of the flour and water, yeast as well as sodium stearoyl lactylate, which is an emulsifier used as dough strengthener in baked goods. After 3 h of fermentation the sponge was mixed with the remaining ingredients, put in forms, and left to rise for 45 min prior to baking. The list of ingredients is given in Table 1. All ingredients were obtained from commercial suppliers in EU except from stone milled fine whole wheat flour and azodicarbonamide, which were obtained from commercial suppliers in US. The whole wheat flour was stone milled and had a protein content of minimum 14%.

Fungamyl Super 7 MA and Novamyl 10.000 BG were both from Novozymes A/S, Denmark. Fungamyl Super is a mix of fungal α-amylase and xylanase and Novamyl is a maltogenic amylase. To prevent microbial growth propanoic acid was added to the dough and post-baking, the bread loaves were treated on the surface with a 0.4 mol l^{-1} potassium sorbate solution. Subsequent to surface treatment the bread was further baked for 1 min to evaporate excess water from the sorbate treatment. The bread was packed for storage in modified atmosphere (nitrogen) after cooling using food grade vacuum plastic bags, 0.09 mm (PA/PE 20/70), from Multiline A/S (Soroe, Denmark) and stored at room temperature. Nitrogen was chosen as filler gas for the purpose of limiting the oxygen availability. Samples were collected over a 5 week period providing samples of dough (just before baking), fresh samples of bread (2 h after baking), and samples stored for 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks. The moisture content in the bread crumb decreased from 44 to 39 g per 100 g during storage whereas it increased in the bread crust from 19 to 31 g per 100 g after 5 weeks of storage. Samples were analysed as triplicates collected from three different bread loaves. Bread loaves were divided into crust and crumb which were further sliced into smaller pieces. Approximately 40 g of sample material was frozen rapidly by immersing samples directly into liquid nitrogen. The frozen bread pieces were grinded and vacuum packed in non-transparent oxygen-impermeable aluminium foil bags (PETP12/ALU9/LLDPE75) from Danisco Flexible (Horsens, Denmark) in aliquots of approximately 3 g and stored at −80 °C for no longer than 70 days prior to analysis.

Table 1
List of ingredients for production of one kilo dough [g].

Ingredients			
Whole wheat flour	538.4	Sodium stearoyl-2-lactylate	2.7
Water	338.9	Ascorbic acid	0.03
Salt	10.8	Calcium propionate	1.3
Glucose syrup	43.1	Mono and di-glycerides	5.4
Cane syrup	21.5	Azodicarbonamide	0.02
Soy oil	10.8	Novamyl 10.000 BG	0.1
Yeast	26.9	Fungamyl Super MA	0.02

2.2. Chemicals

6-Hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (trolox), 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), methanol, chloroform, hexane, acetone, ammonium thiocyanat, iron(III) chloride hexahydrate, fluorescein, ammonium persulphate, and hydrochloric acid were all from Sigma–Aldrich (Steinheim, Germany). Sodium dihydrogenphosphate, barium chloride, iron(II) sulfate, acetic acid, and hydrogen peroxide (30%) were from Merck (Darmstadt, Germany). 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) was purchased from Cayman Chemical Co. (Ann Arbor, MI) and ethyl acetate was from VWR – Bie & Berntsen (Herlev, Denmark). Double de-ionized water was used throughout the experiments.

2.3. TEAC assay

The TEAC assay is based on the scavenging activity of the $ABTS^{\bullet+}$. Extracts for the TEAC analysis were made by dissolving 1 g of sample in 10 ml acetone:water:acetic acid (70:29.5:0.5, v/v). The solution was vortexed for 30 s, extracted for 15 min under agitation, centrifuged for 10 min ($2.1 \times 10^4 \times g$), and the supernatant was collected. The procedure was repeated once more omitting the vortexing step. The joint supernatants were adjusted to a total volume of 25 ml with solvent. ABTS was dissolved in water (19.4 mmol l^{-1}) and mixed with ammonium persulphate (8.8 mmol l^{-1}). The mixture was allowed to react overnight at room temperature in darkness for formation of the $ABTS^{\bullet+}$. Prior to use the $ABTS^{\bullet+}$ solution was diluted 100 times with phosphate buffer (75 mmol l^{-1}, pH 7.4). The TEAC assay was performed in 96 wells transparent microtiter plates from Nunc, Thermo Fisher Scientific (Roskilde, Denmark) by mixing 200 μl of $ABTS^{\bullet+}$ working solution and 50 μl of bread extract or pure solvent (blank). The decrease in absorbance at 414 nm was determined using a Multidetection microplate reader, Synergy™ 2 from BioTek Instruments, Inc. (Winooski, Vermont) and areas under the curve were calculated and converted to trolox equivalents using a standard curve.

2.4. ORAC assay

Extracts used in the ORAC assay were the same as the extracts used in the TEAC. The assay was performed as described by Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) with minor modifications. A fluorescein stock solution was prepared (1.2×10^{-8} mmol l^{-1}) in phosphate buffer (75 mmol l^{-1}, pH 7.4). AAPH was dissolved in phosphate buffer to a final concentration of 15 mmol l^{-1}. The assay was performed using black 96 wells microtiter plates from Nunc, Thermo Fisher Scientific (Roskilde, Denmark). The fluorescein solution (150 μl) mixed with sample extract (25 μl) was incubated at 37 °C for 30 min. Following, the assay was initiated by addition of 25 μl of AAPH solution. The fluorescence was read every minute for 60 min using a fluorescence filter with an excitation wavelength of 485 nm and an emission wavelength of 515 nm using a Multidetection microplate reader, Synergy™ 2. The trolox equivalents were calculated from a standard curve.