Germination of Oat and Quinoa and Evaluation of the Malts as Gluten Free Baking Ingredients

Outi E. Mäkinen · Emanuele Zannini · Elke K. Arendt

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Abstract Germination can be used to improve the sensory and nutritional properties of cereal and pseudocereal grains. Oat and quinoa are rich in minerals, vitamins and fibre while quinoa also contains high amounts of protein of a high nutritional value. In this study, oat and quinoa malts were produced and incorporated in a rice and potato based gluten free formulation. Germination of oat led to a drastic increase of α-amylase activity from 0.3 to 48 U/g, and minor increases in proteolytic and lipolytic activities. Little change was observed in quinoa except a decrease in proteolytic activity from 9.6 to 6.9 U/g. Oat malt addition decreased batter viscosities at both proofing temperature and during heating. These changes led to a decrease in bread density from 0.59 to 0.5 g/ml and the formation of a more open crumb, but overdosing of oat malt deteriorated the product as a result of excessive amylolysis during baking. Quinoa malt had no significant effect on the baking properties due to low α-amylase activity. Despite showing a very different impact on the bread quality, both malts influenced the electrophoretic patterns of rice flour protein similarly. This suggests that malt induced proteolysis does not influence the technological properties of a complex gluten free formulation.

Keywords Pseudocereals · Germination · CLSM · SEM

Abbreviations CD Celiac disease
CLSM Confocal laser scanning microscopy
EFSA European Food Safety Authority
FDA US Food and Drug Administration
FITC Fluorescein isothiocyanate
HPMC Hydroxypropylmethylcellulose
RVA Rapid visco analyzer
SEM Scanning electron microscopy
WPI Whey protein isolate
λex Excitation wavelength

Introduction

Celiac disease (CD) is an immune-mediated enteropathy with a worldwide prevalence of 1 %, triggered by wheat gluten and related proteins in rye and barley. The only treatment is the complete exclusion of these proteins from the diet [1]. Uncontaminated oat is well tolerated by most CD patients and the consumption of “pure oat” is recommended by many national celiac associations, while quinoa is considered safe [2–4]. Commercial gluten free breads are often produced using ingredients low in minerals and fibre [5]. The inclusion of oat and quinoa in standard gluten free diets has been found to improve the nutrient profile, increasing especially the intake of fibre, iron, folate and protein [6].

Oat (Avena sativa) is a cereal rich in minerals, vitamins and compounds with antioxidant activities, and it contains protein high in lysine [7]. Also its main cell wall constituent, (1→3), (1→4)-β-glucan, carries an FDA and EFSA approved health claim on a cholesterol lowering effect [8].

Quinoa (Chenopodium quinoa) is a pseudocereal indigenous to the Andean region, where it has been used as a staple food for thousands of years [9]. Quinoa seeds contain high amounts of vitamins, minerals and protein with a good digestibility and
a balanced amino acid profile [9, 10]. Due to its excellent nutritional value and a potential for production in various climates (incl. Europe), quinoa has been classified as one of the humanity's most promising crops [11, 12].

During malting the grains are soaked, germinated and subjected to a heat treatment to end the metabolic processes and to develop aroma and flavour. During the germination step, the storage compounds of the seed are being mobilised by a variety of synthesised and activated enzymes, resulting in an improved protein digestibility and mineral bioavailability [13, 14]. The metabolic processes occurring in the germinating seeds also lead to an increase in antioxidant activity and the formation of secondary metabolites with possible bioactivities [13, 15].

The aim of this study was to germinate oat and quinoa and evaluate their suitability for use in gluten free baking. In addition to influencing the nutritional profile of gluten free products, the malt enzymes may influence the technological quality of the products: barley and wheat malts are used in the industry as natural dough conditioners in wheat based products. Fortification of wheat bread with unconventional malted grains has been studied previously on malted quinoa, oat, sorghum and brown rice [16–19] but to the authors knowledge no work on gluten free systems has been published.

Materials and Methods

Malting

Oat (variety Lutz, Germany) was steeped, germinated at 15 °C for 5 days and subsequently kilned in three stages (35 °C, 50 °C and 60 °C) in a malting machine (Joe White Malting Systems, Perth, Australia) [18]. Commercial white quinoa (variety unknown, Bolivia) was steeped for 5 h, germinated at 15 °C for 24 h and kilned in four stages (45 °C, 50 °C, 55 °C and 65 °C). Before use the rootlets and protruding cotyledons were removed by hand, and the malts were ground to pass a 0.25 mm sieve.

Enzyme Activities of Malts

α- and β-amylase activities were determined by Ceralpha and Betamyl-3 methods (Megazyme, Wicklow, Ireland). Proteolytic activities were determined from malts extracted in 0.05 M acetic buffer containing 2 mM L-cysteine (pH 5.0) at a ratio of 1:3 for 30 min at 50 °C. Solids were removed by centrifugation (10,000 g x 15 min) and samples assayed against 1.4 % (w/v) azocasein in 0.2 M sodium acetate buffer for 1 h. The reaction was stopped with 10 % trichloroacetic acid and the samples were centrifuged. The supernatant was mixed with 0.5 M NaOH (1:1) and absorbance at 440 nm measured after 20 min. Lipase activities were determined using the dough method [20] by incubating defatted malt samples in a mixture of Tris–HCl buffer (0.05 M, pH 7.5), 9.8 % (w/v) glyceroltrioleate and 1 % Triton-X for 60 min as described in detail previously [17]. The reaction was stopped with 1 M HCl. The free fatty acids were extracted in 2,2,4-trimethylpentane, quantified using the copper soap method against an oleic acid standard curve [21].

Malt Protease Induced Changes in Ingredients Using Lab-on-a-Chip Capillary Electrophoresis

The effect of malt proteases on whey protein isolate (WPI) and rice flour proteins were studied by incubating each ingredient with 5 % malts in 0.2 M acetate buffer (pH 5.4) at 30 °C for 24 h, followed by lyophilisation. Ground samples (rice flour 20 mg; WPI 10 mg) were extracted in 1 ml buffer (5 M urea, 50 mM DTT and 2 % (w/v) SDS in 0.1 M Tris–HCl; pH 8.8) [22]) for 2 h, solids removed by centrifugation (15,000 g x 15 min) and supernatants were diluted (rice flour 1:1 and WPI 1:9). The protein profiles were analysed using a Protein80 kit with a molecular weight range of 5–80 kDa (Bioanalyzer, Agilent Technologies, Palo Alto, USA) under reducing conditions using reagents and standards provided by the manufacturer. For result evaluation the raw data was rescaled to match the height of the upper marker when necessary.

Batter Properties

Starch pasting properties of rice flour and potato starch mixtures (1:1) with oat and quinoa malts were determined using the Rapid Visco Analyzer (General Pasting Method, AACC 76-21). The densities of the batters were measured by transferring 30 g batter in a 100 ml measuring cylinder immediately after mixing and recording the volume before and after 30 min proofing at 30 °C.

For rheological measurements, batters excl. yeast were mixed for 70 s with Glutomatic (Falling Number AB, Huddinge, Sweden), incubated in a proofer (30 °C) for 30 min and then mounted on a controlled stress rheometer (MCR301, Anton Paar GmbH, Austria) with a cross-hatched parallel plate geometry (50 mm; gap 2 mm). A frequency sweep at a 0.01 % strain was performed for angular frequencies (ω) 0.628–62.8 s⁻¹ followed by a viscosity measurement for shear rates 0.6–5 s⁻¹. The complex moduli (G*) values from the frequency sweep were fitted using a weak gel model (Eq. 1) [24].

\[ G^* (ω) = A_F ω^n \] (1)

The effect of the malt enzymes on the viscosities of 0.3 % xanthan and hydroxypropylmethylcellulose (HPC)
solutions was studied by incubating the samples with malt extracts (extracted for 15 min in 0.04 M acetate buffer (pH 4.6); 1:3 extraction ratio) for 30 min at 30 °C and measuring the viscosities between shear rates 1–50 s⁻¹. All measurements were performed at 30 °C.

Baking and Bread Properties

A previously published formulation was used for the baking trials (Online resource 1) [24]. The yeast (2 %; Puratos, Belgium) was activated by dissolving it in 30 °C tap water (90 % on flour basis) and the suspension was added to pre-mixed dry ingredients: rice flour (50 %; Doves Farm Foods Ltd., UK), potato starch (50 %; Doves Farm Foods Ltd., UK), whey protein isolate (10 %; Glanbia, Ireland), vegetable oil (6 %; Homestead, Ireland), sugar (2 %; Siucra, Ireland), salt (2 %; Glacia British Salt Ltd., UK), xanthan gum (0.3 %; Keltrol F; CP Kelko, Atlanta, USA), HPMC (0.3 %; Metolose NE-4000, Harke, Germany) and the ground malts, and mixed for 2 min using a Kenwood Chef (Kenwood Manufacturing Co. Ltd., UK). Batter was proofed in tins for 30 min (30 °C; RH 85 %) and the loaves were baked for 45 min at 190 °C in a deck oven (MIWE, Arnstein, Germany).

The volume of the loaves was measured with VolScan Profiler (Stable Micro Systems, Surrey, UK) and the bread density calculated by dividing the loaf weight by the loaf volume. The crumb hardness was measured using a TAXT2i texture analyser (Stable Micro Systems, Surrey, UK) by compressing a 25 mm slice to 50 % of its original height with a 20 mm aluminium probe. Hardness was defined as the maximum force during compression. Crumb grain was evaluated by image analysis using a C-cell Imaging System and software (Calibre Control International Ltd., UK). The parameters used were cell diameter, wall thickness and number of cells/cm² calculated from the number of cells and slice area.

Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM)

SEM samples were prepared by placing a small drop of batter on stubs (Agar Scientific, plain stubs 10 mm x 10 mm) and immersing the stub in liquid nitrogen after levelling (30 min at 30 °C) or baking at 190 °C. The frozen samples were fractured and lyophilised immediately. Dry samples were mounted on SEM stubs and sputter coated with a 5 μm layer of 80:20 gold-palladium and examined with a JEOL Scanning Electron Microscope (JSM-5510, Jeol Ltd., Tokyo, Japan) at 5 kV and working distance of 20 mm. For CLSM, batters were prepared with a Glutomatic a 1:3 mixture of 0.1 % Rhodamin B and 50 % Calcofluor white (Sigma-Aldrich) as dough liquid, incubated (30 min; 30 °C) and examined with a FV300 confocal laser-scanning system mounted on an Olympus IX80 inverted microscope with a 20x dry objective (Olympus, Germany), using λex=405 and 543 nm. Bread pieces were stained for 5 min in the Rhodamin B and Calcofluor mixture described above, followed by 10 s in 0.3 % FITC (Sigma-Aldrich) in acetone, and rinsed with H2O. The samples were examined using λex=405, 488 and 543 nm.

Statistical Analysis

All analyses were performed at least in triplicates and means were compared using one way analysis of variance with Tukey post-hoc test at a significance level of p<0.05. All statistical analyses were performed using Statistica 7.1 (Statsoft, USA). Model fitting for rheological data was performed using Origin 7.5 (Originlab Corporation, Northampton, USA).

Results and Discussion

Malt Enzyme Activities

The enzyme activities of the malts are given in Table 1. α- and β-amylase activities of oat increased from 0.3 to 48 and 0.5 to 2.3 U/g during malting, respectively. In quinoa malt, the amylolytic activities remained nearly unchanged before and after germination (< 1 U/g). This trend was reflected in the effect of added malts on the RVA peak viscosities: a 0.5 % oat malt addition led to a viscosity loss of 18.5 and 56 % with 2 % oat malt (Table 2). Quinoa malt decreased the peak viscosity only little: 2.5 and 5 % quinoa malt additions decreased the viscosities by 4.7 and 5.2 %, respectively. A starch paste viscosity (Amylograph) loss of 18 % as a result of adding 5 % 12 h germinated quinoa in wheat flour has been reported previously [25]. The low amylolytic activities observed in this study may be caused

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Enzyme activities of oat and quinoa before and after malting (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>α-amylase a</td>
</tr>
<tr>
<td>Oat</td>
<td>0.29±0.07 b</td>
</tr>
<tr>
<td>Oat malt</td>
<td>47.7±2.0 a</td>
</tr>
<tr>
<td>Quinoa</td>
<td>0.09±0.05 b</td>
</tr>
<tr>
<td>Quinoa malt</td>
<td>0.08±0.01 b</td>
</tr>
</tbody>
</table>

a Unit of activity: 1 μmol p-nitrophenol min⁻¹ at pH 5.2, T=40 °C
b Unit of activity: liberation of 1 μmol oleic acid/h at pH 7.1 and 37 °C
c Unit of activity: increase in A440nm/h at pH 5.4; 40 °C
by varietal differences or poor germination performance due to post-harvest processing.

Lipase activity of unmalted oat was high, and malting only slightly increased it (Table 2). No lipase activity was detected in unmalted quinoa, but a low level of activity (1.3 U/g) appeared as a result of malting. The protease activity of oat nearly tripled to 5.68 U/g during malting, but decreased from 9.6 to 6.9 U/g in quinoa. Compared to barley and wheat malts, both malts produced in this study were high in proteolytic activities [17].

Malt Protease Induced Changes in Ingredients

The electropherograms of the WPI samples revealed two peaks at 15 kDa and 25 kDa, but malt proteases had no visible effect on either of the peaks (not shown). The electropherograms of rice flour protein (Fig. 1) show major peaks at molecular weights 10–15 kDa (a prolamin), at 21 kDa and a triple peak at 36–39 kDa (corresponding to oryzenin subunits) and two peaks at 55 and 59 kDa [26]. After 24 h, all peaks except the last one (59 kDa) showed a decrease in samples digested with both oat (Fig. 1a) and quinoa malts (Fig. 1b). No differences between the oat malt and quinoa malt digested samples were observable.

Table 2 Properties of batters with oat (O) and quinoa (Q) malts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RVA</th>
<th>Rheology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak viscosity (RVU)</td>
<td>Viscosity loss (%)</td>
</tr>
<tr>
<td>Control</td>
<td>363±2.2 a</td>
<td>0.0</td>
</tr>
<tr>
<td>O 0.5 %</td>
<td>296±2.8 c</td>
<td>18.5</td>
</tr>
<tr>
<td>O 0.75 %</td>
<td>256±3.1 d</td>
<td>29.5</td>
</tr>
<tr>
<td>O 1 %</td>
<td>225±3.2 e</td>
<td>37.9</td>
</tr>
<tr>
<td>O 2 %</td>
<td>159±8.3 f</td>
<td>56.2</td>
</tr>
<tr>
<td>Q 1 %</td>
<td>356±3.4 ab</td>
<td>1.8</td>
</tr>
<tr>
<td>Q 2.5 %</td>
<td>346±12.4 b</td>
<td>4.7</td>
</tr>
<tr>
<td>Q 5 %</td>
<td>344±0.9 b</td>
<td>5.2</td>
</tr>
</tbody>
</table>

$r^2$ was > 0.95 for all samples

The electropherograms of rice flour protein (Fig. 1) show major peaks at molecular weights 10–15 kDa (a prolamin), at 21 kDa and a triple peak at 36–39 kDa (corresponding to oryzenin subunits) and two peaks at 55 and 59 kDa [26]. After 24 h, all peaks except the last one (59 kDa) showed a decrease in samples digested with both oat (Fig. 1a) and quinoa malts (Fig. 1b). No differences between the oat malt and quinoa malt digested samples were observable.

Batter Rheology

Only 2% oat malt decreased batter viscosities significantly (Table 2). The results from the small strain oscillation measurements were interpreted by fitting the data in a weak gel model, yielding parameters $A_F$ and $z$. The $z$ value is an interaction factor indicating the amount of interactions in the food network, while $A_F$ can be interpreted as the strength of the interactions [23]. As a result of oat malt addition the $A_F$ values decreased, while $z$ values remained nearly unchanged. This suggests that the amount of interactions remained the same while only their strength was weakened as a result of oat malt enzyme action on the batter components, as opposed to the effect of sourdough fermentation that decreases both factors [27]. The viscosities of 0.3% xanthan gum and HPMC solutions were 1.4 and 0.01 Pas at 5 s$^{-1}$, respectively, and not influenced by incubation with malt enzyme extracts (not shown).

As starch is still in its granular state at the proofing temperature and not prone to amylolysis, the main contributors to the rheological properties of the batters were xanthan gum and proteins (endogenous and ingredient derived). As oat malt had no influence on the viscosity of a xanthan gum solution, it would appear that the viscosity decreasing effect would be due to proteolysis, as reported in previous studies [28, 29]. However, quinoa malt had a proteolytic
activity comparable to oat malt and their effect on the electrophoretic pattern of protein containing ingredients was identical. The impact of proteolysis on gluten free batter and bread properties depends strongly on the matrix [29]. A possible explanation for the apparent lack of significance of the proteolytic activities may be the dominant effect of two ingredients with strong foaming properties, HPMC and WPI.

Bread Properties

Loaves with varying levels of oat (0.5; 0.75; 1 and 2 %) and quinoa (1 %; 2.5 % and 5 %) malts were baked in addition to a control bread without malt. The addition of oat malt decreased the bread densities (Table 3), translating to higher loaf volumes, but a 2 % addition led to a formation of large holes in the centre of the crumb (Online Resource 2). There were no significant differences in batter densities between the control and batters with oat malt. Quinoa malt addition had no impact on bread or batter densities even at an addition level of 5 %. Image analysis showed that oat malt addition gave a more open crumb with fewer and larger cells with thicker walls. The no. cells/cm² decreased from 80.8 to 63.9 in the bread baked with 0.75 % oat malt, still resulting in an even crumb. The cause for the lower values in the breads with 1 and 2 % oat malt is the forming of large holes due to excessive cell coalescence in the centre of the loaf. Quinoa malt had no significant effect on the crumb grain.

Batter densities showed no differences but the densities of baked breads decreased with increasing oat malt level. It would thus appear that the changes leading to a higher loaf volume and more open crumb grain occurred during baking. RVA results indicated a drastic drop in peak viscosity as a result of oat malt α-amylase action, which has a major impact on the stability of the gas cells. The lack of impact of quinoa malt on any bread properties is probably due to a very low α-amylase activity. This also suggests that proteolytic activity had little role in the properties of the formulation used in this study, as both malts contained high protease activities.

Malt additions had no influence on crumb hardness (not shown). A crumb softening effect in rice bread using a maltogenic α-amylase has been reported before [30]. Possibly the early inactivation of malt α-amylases (75–80 °C) makes them inefficient as crumb softening enzymes. Lipases alter the polarity of lipids that may contribute to the stability of the gas cell walls [31]. Monoglycerides have been reported to increase the volume and increase the cell size in the crumb when added in the same formulation used in this study [24]. Lipases may thus influence the bread properties, but their role was not confirmed in this study.

Microscopy

CLSM micrographs of bread crumbs (Fig. 2) show a dominating matrix of gelatinised starch visualised with FITC, surrounded by discontinuous networks of protein and

### Table 3 Bread and batter densities and crumb properties with oat (O) and quinoa (Q) malts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Batter density (g/ml)</th>
<th>Bread density (g/ml)</th>
<th>No. cells/cm²</th>
<th>Cell diameter (mm)</th>
<th>Wall thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69±0.03 a</td>
<td>0.59±0.02 a</td>
<td>80.8±7.5 a</td>
<td>1.55±0.21 a</td>
<td>0.44±0.01 a</td>
</tr>
<tr>
<td>O 0.5 %</td>
<td>0.66±0.04 a</td>
<td>0.58±0.04 ab</td>
<td>71.4±8.6 b</td>
<td>1.89±0.35 b</td>
<td>0.48±0.03 b</td>
</tr>
<tr>
<td>O 0.75 %</td>
<td>0.67±0.03 a</td>
<td>0.54±0.03 bc</td>
<td>63.9±5.8 cd</td>
<td>2.27±0.26 c</td>
<td>0.51±0.01 c</td>
</tr>
<tr>
<td>O 1 %</td>
<td>0.69±0.04 a</td>
<td>0.53±0.02 c</td>
<td>64.7±3.9 c</td>
<td>2.28±0.18 c</td>
<td>0.51±0.01 c</td>
</tr>
<tr>
<td>O 2 %</td>
<td>0.67±0.02 a</td>
<td>0.50±0.01 d</td>
<td>56.0±4.1 d</td>
<td>2.63±0.25 d</td>
<td>0.50±0.01 d</td>
</tr>
<tr>
<td>Q 1 %</td>
<td>0.70±0.03 a</td>
<td>0.60±0.02 a</td>
<td>79.0±6.4 ab</td>
<td>1.45±0.13 a</td>
<td>0.43±0.01 a</td>
</tr>
<tr>
<td>Q 2.5 %</td>
<td>0.70±0.02 a</td>
<td>0.58±0.04 a</td>
<td>79.2±9.5 ab</td>
<td>1.55±0.31 a</td>
<td>0.44±0.02 a</td>
</tr>
<tr>
<td>Q 5 %</td>
<td>0.69±0.02 a</td>
<td>0.58±0.04 a</td>
<td>78.8±8.9 ab</td>
<td>1.57±0.26 a</td>
<td>0.45±0.02 ab</td>
</tr>
</tbody>
</table>

Fig. 2 CLSM micrographs of bread crumbs: control (a); 2 % oat malt (b) and 2.5 % quinoa malt (c). Letters indicating protein (P), starch (S) and hydrocolloids (H); Bars 200 μm.
hydrocolloids. The protein matrix consists of larger aggregates in the control bread compared to the ones baked with malts. CLSM micrographs of the batters and SEM micrographs of batters and breads reveal no visible differences upon malt addition (Online resource 3).

Conclusion

Oat malt produced from pure oats may be used to improve the volume and crumb grain of gluten free bread at levels <1 %, but overdosing may deteriorate the crumb. Quinoa malt had no effect on the baking quality and germinating quinoa for bakery products may not be feasible, unless improvements in the palatability and nutritional properties are desired. The key parameter to altered technological properties was α-amylase activity. Malt induced proteolysis did not influence the properties of a gluten free formulation at least in the presence of other ingredients with strong foaming properties such as WPI and HPMC.

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