Changes in Wheat Grain Quality Due to Doubling the Level of Atmospheric CO_2

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ABSTRACT

Elevated levels of atmospheric CO_2 have been shown to increase grain yield and reduce grain nitrogen concentration. The object of this study was to determine whether elevated CO_2 levels would modify other aspects of grain quality relevant to processing, particularly protein and starch quality. Wheat of two genotypes (Hartog and Late Hartog) was grown in the field in controlled-atmosphere tunnels at either the ambient level of CO_2 (350 μl/L) or an elevated level (700 μl/L). This elevated level of CO_2 produced significant increases in grain yield, but decreases in 1,000-kernel weight. Grain grown in the elevated CO_2 atmosphere produced poorer dough and decreased loaf volume, farinograph development time, and dough extensibility. These changes were largely attributable to the lower protein content of the grain grown at elevated CO_2. There did not appear to be major changes in protein composition or in the functional properties of the protein. Grain produced at elevated CO_2 yielded starch with a significantly higher proportion of large (A-type) starch granules but no overall change in amylose-to-amylopectin ratio. These studies indicate that elevated levels of CO_2 may result in decreased quality of bread wheats largely due to lowered protein content.

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By the middle of the next century, atmospheric CO_2 levels are predicted to double from their present level of 350 μl/L. This has important implications for agriculture (Conway et al 1988, Adams et al 1990, Conroy 1992, Wigley and Raper 1992, Bowes 1993, Conroy et al 1994). Global climate models have indicated that there will be a concomitant increase in temperature, coupled with increased variability in daily temperatures (Adams et al 1990, Wigley and Raper 1992, Bowes 1993). Studies of the consequent effects on wheat, one of the world's major temperate cereals, have largely been limited to the effects on plant biomass and grain yield. For example, an early study of dwarf spring wheat, grown in enclosed chambers in air enriched with CO_2 to 750 μl/L (Fischer and Aguilar 1976), indicated an average increase in plant dry weight of 8%. Grain yield (grains/m^2) increased by up to 23% with the extra CO_2, due to increases in spike number or grains per spikelet. When grain yield increased, kernel weight decreased, presumably as a result of increased intergrain competition (see also Havelka et al 1984). In a similar study, Mitchell et al (1993) also found an increase in total dry-matter production with elevated CO_2 (692 μl/L) and a stimulation of grain yield when nitrogen was not limiting. With limited nitrogen supply at ambient temperatures, there was a significant decrease in grain yield.

Field trials examining high CO_2 effects have the potential to provide results that will be more realistic than glasshouse experiments for extension to commercial practice, but the design of field trials present experimental difficulties. These problems have been largely overcome by the introduction of tunnels to enclose field-grown plants in a stream of air containing an elevated level of CO_2 (Rawson et al 1995). Wheat grown with elevated CO_2 (enriched to 700 μl/L) (Rawson 1995) increased grain yield by up to 36% for summer plantings. Yield increases were much lower (averaging 7%) for lower growth temperatures.

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What implications do these results have for the wheat processing industries? Wheat grain, grown at elevated CO_2 (700 μl/L) in a glasshouse has been reported to show a reduction in protein content that was disproportionately greater than the increase in yield (Thompson and Woodward 1994). Conroy et al (1994) also reported lower protein content for wheat (cv. Hartog, based on flour analysis) grown in the glasshouse. They attributed this reduction to the observed changes in leaf nitrogen and alanine levels (up to 50% lower when grown in elevated CO_2 atmospheres of either 550 or 900 μl/L).

If elevated CO_2 is likely to lead to a reduction in grain protein content, this trend would have serious consequences for the wheat processing industry, since protein content is a major determinant of grain prices and processing quality. Also important are the possible effects of CO_2 "fertilizer" on the qualitative aspects of grain composition, particularly protein quality, as well as starch and lipids. Tester et al (1995) recently reported on field experiments in tunnels with ambient and elevated levels of CO_2, but there were "no consistent major effects on any of the parameters measured" (including starch content and starch properties) due to doubling the level of CO_2. Increases in wheat grain lipids have been reported for wheat grown at elevated CO_2 (700 μl/L) (Williams et al 1993), but there was no information about the overall effects of high CO_2 on processing quality. Subsequently, these authors (Williams et al 1995) reported that effects on lipids due to elevated CO_2 were outweighed by (and interactive with) elevation of growth temperature.

We have, therefore, investigated these attributes for a series of grain samples grown in the field in tunnels with ambient air or air enriched in CO_2 to a level of 700 μl/L. Most evident was a drop in grain protein content, changes in dough and baking qualities, and an increase in the proportion of large (A-type) starch granules. Furthermore, this study of the effects of CO_2 on quality may lead to a new insight into the regulation of grain composition and, by that new knowledge, to new opportunities to manipulate grain quality.

MATERIALS AND METHODS

Grain Samples

Grain of the bread wheat Hartog (H) and its near isoline Late Hartog (LH) were produced in the field in the Canberra district (ACT, Australia), either at the Black Mountain site or at the Gin-

ninderra Experiment Station (Table I). LH (Sun 224A) is an isoline of H, being derived from three backcrosses to H (Rawson and Zajac 1993). Grain from a December 1991 planting (Rawson 1995) was not analyzed in this study of grain quality.

In three field trials, the plants were covered during growth by clear plastic tunnels with dimensions of 8 × 1.25 × 1.25 m (length × width × height) (Rawson 1995). Air blown through the tunnels provided either an ambient level of CO_2 (350 µl/L) or an elevated level of CO_2 by enrichment of the air passing through the second set of tunnels. The field sites were irrigated to provide replacement of measured evaporation. Details of wheat varieties, soil types, and management are provided by Rawson (1995).

As a result of the range of sowing times adopted, mean daily maximum temperatures during grain filling ranged from 25 to 32°C (Table I). Other factors, such as solar radiation (also detailed by Rawson [1995]), varied among the trials. The temperature in the tunnels was slightly higher than ambient, but never more than 2°C higher throughout the growing season. There was a small gradient of temperature (averaging <2°C) along each tunnel, but the effect on grain quality analysis was minimized by appropriate blending of grain samples, yielding either two or three samples of ≈300 g each (Table I), from each of the three trials for each combination of CO_2 treatment and variety, i.e., a total of 32 grain samples.

Grain and Flour Analyses

These 32 grain samples were analyzed (replicated in duplicate or more) for 1,000-kernel weight (TKW) and grain hardness by an adaptation of the particle size index test of Symes (1961). Grain was conditioned to 16% mc before milling to flour in a Quadrumat Junior mill (Brabender, Germany). Protein content was determined on flour by the Dumas method (Trials 1 and 2) or near infrared reflectance (NIR) (Trial 3). Flour samples were tested for dough properties in the 2-g mixograph (Rath et al 1990) with analyses replicated three times, followed by computer-based interpretation. Results were expressed as time to peak (mix time, in seconds), resistance breakdown (as the percentage drop in resistance, 3 min after the peak), and as the height at peak resistance (in arbitrary units).

Dough properties were analyzed (in duplicate or more) using the farinograph (AACC method 54-21, AACC 1995) to give dough-development time, and in the extensigraph (small-scale modification of the RACI method [1988]) to give curve height (R_{max}), for the first two seasons by the Bread Research Institute of Australia, and for the last season by the NSW Agricultural Research Centre in Tamworth, NSW, Australia.

TABLE I
Growth Conditions for Grain Samples and Yield Results[a]

Conditions	Trial 1 (Summer)	Trial 2 (Spring)	Trial 3 (Winter)
Site	Ginninderra	Black Mountain	Ginninderra
Date planted	17 Dec, 1992	2 July, 1992	29 April, 1993
Date harvested	1 April, 1993	20 Dec, 1992	29 Nov, 1993
Mean temperatures (°C)			
Preanthesis	22.7	10.5	9.7
Postanthesis	20.4	17.2	15.0
Minimum temperatures (°C)			
Preanthesis	15.8	5.5	4.3
Postanthesis	13.1	11.2	7.9
Maximum temperatures (°C)			
Preanthesis	36.1	20.0	19.3
Postanthesis	31.9	27.3	24.9
Mean grain-yield differences due to CO_2			
Hartog	+36%	+12%	+11%
Late Hartog	+34%	+5%	+1%
Replicates	3	2	3

[a] For two wheat varieties (Hartog and Late Hartog) grown at two CO_2 levels: ambient and elevated (750 µl/L) (Rawson 1995).

Baking Properties

In Trial 3, there was sufficient grain to permit test baking. This was performed according to the standard method of the Agricultural Research Centre in Tamworth, NSW, Australia, using 110-g flour samples in a rapid-dough method without bromate. The dough formula contained (on a flour-mass basis) 2.5% compressed yeast, 2% sodium chloride, 1% ammonium chloride, and 0.6% malt extract. Water absorption and mixing time (2–4 min) were based on farinograph testing.

Protein Composition

The ratio of glutenin to gliadin (Glu-Gli) was determined by size-exclusion high-performance liquid chromatography (SE-HPLC) by the method of Batey et al (1991), using the protein extracted from flour without reducing agent by sonication in phosphate buffer containing sodium dodecyl sulfate (SDS). The major peak eluting first was defined as aggregated glutenin and the second major peak as monomeric gliadin. (Qualitatively similar elution profiles were reported by Blumenthal et al [1994]).

The proportion of unextractable polymeric flour protein as a ratio of extractable flour protein (in 0.5% SDS solution) was also examined (Gupta et al 1993) as this has been highly correlated with dough strength properties. Extractable and unextractable protein was determined by SE-HPLC of extracts in 0.5% SDS-phosphate buffer.

Starch Properties

Freeze-dried starch was analyzed for particle-size distribution in a laser diffraction particle sizer (series 2600, from Malvern Instruments, Malvern, Worcestershire, U.K.), as described by Blumenthal et al (1995). Results were reported as the percentage of B-type starch granules (<10 µm) by volume (%B granules). Granules >10 µm were classed as A-type granules. The particle-size distribution of starch granules isolated from flour samples was determined by dispersing 100 mg of flour in 0.5*M* sodium chloride solution, resting it at 4°C for 45 min, and kneading it in saline to separate the starch from the gluten ball. The kneading in 0.5*M* sodium chloride solution was repeated three times. The combined washings of starch were centrifuged, washed twice by suspension in 0.1*M* acetic acid solution, and finally suspended in water.

The effect of elevated CO_2 on starch structure was investigated by determining the degree of branching and the amylose-to-amylopectin ratio (using α-amylase or isoamylase digestion, respectively, on starch freeze-dried as described above) (Blumenthal et al 1994).

TABLE II
Grain Quality Attributes: Mean Values and Least Significant Difference (LSD) for All Grain Samples

	Ambient CO_2 (350 µl/L)	Elevated CO_2 (700 µl/L)	LSD
Yield (g/m^2)[a]	689.3	809.3	na[b]
TKW (g)[c]	41.9	38.3	2.7
Protein (%,flour)	10.1	8.7	0.4
Mix time (sec)	264	243	38 ns[d]
Peak resistance	296	249	11
Breakdown (%)	14	13.4	2.2 ns
%B granules	26.2	22.0	1.3
Glu/Gli ratio	1.12	1.19	0.01

[a] Yield results (Rawson 1995).
[b] Not available.
[c] 1,000 kernel weight.
[d] Not significant.

Lipid Analysis

Duplicate determinations of the levels of *N*-hexane-extractable free lipids (FL) and of water-saturated *n*-butanol bound lipids (BL) were conducted on each of the 32 flour samples, in duplicate, using 500 mg of flour, according to Bekes et al (1983).

Statistical Analyses

Results were analyzed for statistical significance by analysis of variance or by correlation, as appropriate, using standard algorithms provided by the MSUSTAT package of statistical programs, Version 4.11. MSUSTAT was developed by R. E. Lund, Montana State University, Bozeman, MT.

RESULTS AND DISCUSSION

Grain Yield and Kernel Size

Treatment with an elevated level of CO_2 (700 µl/L) caused general increases in grain yield when compared with grain grown under ambient conditions (Table II) (Rawson 1995). This stimulation of yield by elevated CO_2 was considerable under the warmer conditions, especially those used in Trial 1 (summer) with maximum temperatures of 36.1 and 31.9°C before and after flowering, respectively (+36% and +34%, for H and LH, respectively, in Trial 1). The increase in yield associated with CO_2 was more moderate at lower temperatures (+11% and +1%, for H and LH, respectively, in Trial 3; maximum temperature postanthesis was 24.9°C). Rawson (1995) has discussed the possible reasons for differences between the genotypes.

The increases in yield associated with elevated CO_2 are likely to be due to factors such as numbers of grains and tillers (Rawson 1995), because CO_2 enrichment caused a decrease in kernel weight (Table II). There were significant differences in TKW (Tables III and IV) for the bulking of grain samples used in our analyses from the results in Rawson (1995). Three-factor analysis of variance was used to analyze the overall effects of CO_2 treatment, genotype, and growth trial on kernel weight. This analysis showed that only one of these factors was significant: CO_2 level ($P = 0.01$). The effects of genotype and growth trial were much less significant ($P = 0.48$ and 0.11, respectively). There were significant interactions among all main effects. Within the results for the specific trials (Tables III and IV), the significant changes in kernel weight were confined to Trials 1 and 3 ($P = 0.05$ and <0.001 for CO_2), but these provided the best comparison as they were conducted under more similar conditions (both at Ginninderra).

Protein Content and Composition

Three-factor analysis of variance showed two significant effects on flour-protein content: 1) effect of elevated CO_2 ($P < 0.001$) and trial ($P < 0.001$), and 2) CO_2 × trial interactions (Table V). The samples from Trials 1 and 3 had higher protein contents than those from Trial 2 (Tables III and IV). There was no significant correlation between mean growth temperature and protein content, so the differences probably relate to nutritional differences between sites. In each trial, there was a reduction in protein content as a result of doubling the level of CO_2.

The ratio of aggregated glutenin to monomeric gliadin (Glu-Gli) varied significantly (Tables II, III, and IV) under the influence of all three factors, ($P = <0.001$, 0.07, and <0.001 for CO_2, genotype, and trial, respectively). Overall, Glu-Gli was closely related to flour-protein content ($r = -0.91$).

For samples from Trial 2, elevated CO_2 had no significant effect on the proportions of unextractable and extractable glutenin protein ($P = 0.62$).

Dough Properties

According to mixograph analyses, elevated CO_2 did not cause significant (or consistent) changes in the dough attributes of mixing time to peak, with the exception of Trial 3 (Tables III and IV) or in resistance breakdown after the peak (Tables II, III, and IV), but there was a considerable (14%) decrease in peak resistance associated with all three factors ($P < 0.001$ for CO_2, cultivar, and trial). This decrease was presumably due to the close relationship of peak resistance to protein content, which showed comparable decreases.

TABLE III
Grain Quality Attributes: Averages Comparing CO_2 Levels

	Trial 1			Trial 2			Trial 3		
	Ambient	Elevated	Significance	Ambient	Elevated	Significance	Ambient	Elevated	Significance
TKW (g)[a]	44.3	39.2	$P < 0.05$	42.0	38.8	ns[b]	39.5	37.8	$P < 0.01$
% Protein	10.9	8.8	$P < 0.01$	7.1	7.0	ns	12.3	10.4	$P < 0.01$
Mix time (sec)	198	218	ns	267	238	ns	269	336	$P < 0.05$
Peak Resistance[c]	398	319	$P < 0.01$	311	267	$P < 0.01$	180	151	$P < 0.01$
Breakdown (%)	11.8	10.1	ns	13.4	14.4	ns	16.8	15.6	ns
%B granules	24.1	21.5	ns	28.9	24.0	$P < 0.05$	24.9	20.6	$P < 0.05$
Glu-Gli ratio	1.06	1.20	$P < 0.001$	1.51	1.57	$P < 0.01$	0.81	0.80	ns

[a] 1,000 kernel weight.
[b] Not significant.
[c] Arbitrary units.

TABLE IV
Grain Quality Attributes: Averages Comparing Genotypes

	Trial 1			Trial 2			Trial 3		
	Hartog	Late Hartog	Significance	Hartog	Late Hartog	Significance	Hartog	Late Hartog	Significance
TKW (g)[a]	42.9	40.6	ns[b]	39.9	41.4	ns	39.7	37.5	$P < 0.001$
% Protein	10.4	9.3	$P < 0.05$	7.0	7.1	ns	11.4	11.4	ns
Mix time (sec)	213	203	ns	167	338	$P < 0.001$	338	266	$P < 0.05$
Peak resistance[c]	381	336	$P < 0.01$	320	258	$P < 0.001$	174	158	$P < 0.05$
Breakdown (%)	7.6	14.3	$P < 0.05$	12	15.8	ns	12.3	20.1	$P < 0.001$
%B granules	20.7	24.9	$P < 0.05$	26.9	26	ns	22.7	22.8	ns
Glu-Gli ratio	1.07	1.20	$P < 0.001$	1.59	1.49	$P < 0.001$	0.80	0.81	ns

[a] 1,000 kernel weight.
[b] Not significant.
[c] Arbitrary units.

Sufficient grain was obtained from the Trial 3 to perform physical dough testing and baking trials (Table VI). Samples from the elevated CO_2 treatment showed significant reductions in loaf volume, dough extensibility, and dough-development time in the farinograph, for both H and LH. Changes in extensigraph R_{max} and in water absorption were not statistically significant. The changes in loaf volume, dough extensibility, and dough-development time were attributable to, and commensurate with, the changes observed in protein content. For the grain from Trial 3 (at least), there was a general decrease in processing quality for baking, although again this change could be associated largely with the decrease in protein content for grain grown in an elevated CO_2 atmosphere.

Starch Properties

Both elevated CO_2 and trial generally affected the proportion by volume of small (B-type) starch granules (Tables II, III, and IV), giving a considerable decrease (absolute difference of 4%, $P < 0.001$) in the proportion of small granules with elevated CO_2. Increasing temperatures >32°C during grain filling have been reported to cause a decrease in the proportion of small granules (Blumenthal et al 1994), but the effects of temperature <32°C are not well characterized. Grain from Trial 1, with the highest postanthesis temperature (32°C maximum), had the lowest volume proportion of B-granules. This result may indicate that moderate temperatures also affect granule-size distribution. Overall, there were no varietal differences evident with regard to the proportion of B-type starch granules ($P = 0.26$), but in Trial 1, the proportion of B granules was significantly lower for the earlier-maturing cultivar (Tables III and IV).

No significant differences were evident in either total starch content or starch structure (determined as amylose-to-amylopectin ratio) as a consequence of elevated CO_2 treatment ($P = 0.67$ and 0.88, respectively).

Lipids

Elevated concentrations of CO_2 had no significant effect ($P > 0.05$) on levels of total, free, or bound lipids, although there was a significant difference between H (3.8% bound lipid) and LH (4.3%) in the levels of bound lipids ($P = 0.05$), irrespective of growth conditions.

TABLE V
Three-Factor Analysis of Variance for Effects on Flour Protein Content

Source	Degrees of Freedom	Sums of Squares	*P*-Value
Trial	2	101.00	<0.001
CO_2	1	14.00	<0.001
Trial × CO_2	2	6.02	0.002
Genotype	1	0.901	0.123
Trial × Genotype	2	2.10	0.071
CO_2 × Genotype	1	1.05	0.097
Trial × CO_2 × Genotype	2	1.53	0.136
Residual	20	6.94	

TABLE VI
Effects of Levels of CO_2 on Baking and Dough Quality[a]

	Loaf Volume (ml)	Dough Extensibility (cm)	Dough Development Time (sec)
Hartog			
Ambient	695	17.7	8.8
Elevated	598	15.9	1.8
Late Hartog			
Ambient	633	16.4	3.3
Elevated	583	14.8	1.8
Both genotypes			
Ambient	664	17.1	6.0
Elevated	591	15.4	1.8
P-values for elevated CO_2	0.02	0.01	<0.001

[a] From Trial 3 only. All values represent means of triplicate determinations.

Implications of the Results

Should the concentration of CO_2 in the atmosphere rise during coming decades, a decrease in grain nitrogen and an increase in grain yield can be expected for wheat. Since this predicted rise in CO_2 is likely to be accompanied by temperature increases (the scenario generally described), the effect of CO_2 on grain protein content may be partially balanced because temperature increases can elevate grain-protein content (Randall and Moss 1990, Wrigley et al 1994). However, the influence of high CO_2 on plant, and particularly grain, nitrogen is not merely a growth dilution effect. Therefore, it is unlikely that any high-temperature effects will totally compensate for elevated concentrations of CO_2 in the atmosphere.

These results suggest that the expected changes in protein content are likely to be more significant than major changes in the functional properties of the protein for the processing of wheats in the future. For starch, it appears likely that the combined effects of higher CO_2 levels and higher temperatures will decrease the proportion of B-type starch granules and increase the proportion of the desirable large A-type starch granules. This change could offer advantages to some processors, especially for starch-gluten production, with no obvious disadvantages in other aspects of wheat utilization.

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