

Influence of the Farming System on the Xanthophyll Content of Soft and Hard Wheat

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The organic market is growing rapidly. This is because customers expect organic food to be authentic and healthy. For plant products the awareness of pesticide residues is one main point in customers' decisions for organic food, but in terms of secondary plant compounds, antioxidants are also expected benefits of organically produced foods. For wheat the xanthophylls are one group of those secondary plant compounds. There are no recent studies about the influence of cultivation practices on the xanthophyll content. This study examined the influence of the farming system on the content of lutein and zeaxanthin. To evaluate this, samples of a long-term field trial were examined by comparing conventional (nonorganic) and organic produce grown under controlled conditions. Additionally, samples were examined from farm pairs located in Germany. Each of the pairs consisted of one organically and one conventionally producing farm, located in local neighborhood and cultivating the same wheat variety. To summarize, the influence of the farming system is very small. The differences are mainly caused by different kernel sizes (thousand-kernel weight), which are found to be correlated to the lutein content.

KEYWORDS: Xanthophylls; organic agriculture; wheat; lutein

INTRODUCTION

The organic market is growing rapidly (1). The reasons for customers' decisions to buy organic food are very complex. Bonti-Ankomah and Yiridoe describe a detailed view of the consumer perception theory for the organic market (2). Besides the awareness of pesticide residues in food, the opinion that organic food may have the advantages of a health-supporting nutrition is one of the main reasons for buying organic food.

In wheat, secondary plant compounds such as xanthophylls, tocopherols, and phenolic compounds (3) are strongly influenced by variety and growing conditions (3–5). Lutein is the major carotenoid in wheat (6). It was shown that lutein prevents the wheat embryo from aging (7). Therefore, it is primarily located in the aleuronic layer of the kernel and the embryo (6). Its function in human physiology is described (9, 10). Various studies compared the nutritional value of organically and conventionally produced crops, fruits, and vegetables (11–13), but no major differences were found except for slightly higher vitamin C and dry matter contents in some plant products from organic produce.

The definitions for "organic agriculture" (14, 15) can be reduced to two main differences between organic and conven-

tional produce. These differences are mainly related to the use of some mineral fertilizers and synthetic pesticides. The use of these inputs is prohibited for organic production (16). Due to regulations and limitations in fertilizer availability, the mean intensity of fertilization (expressed as kg/ha) is less in organic farming (17, 18). However, there is a huge variance in farming practices, and an overlapping of some cultivation conditions cannot be ruled out. To study the effects of the cultivation system, long-term field trials were set up (17–20). These experiments represent typical cultivation practices including fertilization and weed management as used in practice. In addition, samples derived from neighboring farms using organic and conventional methods can be used as samples from practice. Here, the influences of environmental factors (e.g. soil and climate) and variety have to be taken into account. In particular, the varieties cultivated under organic farming system differ from those cultivated in conventional farming, particularly (21).

There is very little knowledge of the influence of various factors on the lutein content of wheat published. In the past only screenings of the lutein content over species and varieties were done (3, 5, 10, 22–24). Therefore, the goal of these studies was to examine mainly the genetic variability, which was found to be very high. For soft wheat, concentrations from below 1 to 4 $\mu\text{g g}^{-1}$ relating to the fresh matter were found, for example.

In only one study was the influence of the location analyzed. In a cross-over study with samples of one year and two soft wheat varieties grown at two locations no consistent influence of the location-related conditions was found (25). Extended

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Table 1. Main Characteristics of the Compared Systems in the DOC Trial

	practice				
	organic farming system		conventional farming systems ^a		unfertilized
	biodynamic (BIODYN)	bio-organic (BIOORG)	conventional with farmyard manure (CONFYM)	conventional solely mineral fertilizers (CONMIN)	like BIODYN but unfertilized (NOFERT)
type and level	composted farmyard manure (FYM) and slurry 0.4 LU ha ⁻¹ year ^{-1b}	rotted FYM and slurry 1.4 LU ha ⁻¹ year ^{-1b}	Fertilization stacked FYM and slurry 1.4 LU ha ⁻¹ year ^{-1b} plus mineral fertilizer up to a level according to official guidelines	only mineral fertilization according to official guidelines	unfertilized
weed control	mechanical	mechanical	Plant Protection mechanical and herbicides	mechanical and herbicides	mechanical
disease control	indirect methods	indirect methods, copper	fungicides (thresholds)	fungicides (thresholds)	indirect methods
insect control	plant extracts, biocontrol	plant extracts, biocontrol	insecticides (thresholds)	insecticides (thresholds)	plant extracts, biocontrol
special treatments	biodynamic preparations	none	plant growth regulators	plant growth regulators	biodynamic preparations

^a Referred to conventional, although they have been managed as integrated systems since 1985. ^b LU, livestock units. The fertilization was typical of Swiss organic farms. Table is slightly modified from ref 18.

Table 2. Main Characteristics of the Organic and Conventional Variants of the Hard and Soft Wheat Cultivation in the MASCOT Experiment^a

	soft wheat (variety 'Bolero')		hard wheat (variety 'Claudio')	
	conventional	organic	conventional	organic
abbreviation	TAC	TAO	TDC	TDO
	mineral 156 kg ha ⁻¹ N 92 kg ha ⁻¹ P ₂ O ₅ 30 kg ha ⁻¹ K ₂ O	Fertilization; type and level organic ^a 30 kg ha ⁻¹ N 30 kg ha ⁻¹ P ₂ O ₅ 30 kg ha ⁻¹ K ₂ O	mineral 156 kg ha ⁻¹ N 92 kg ha ⁻¹ P ₂ O ₅ 0 kg ha ⁻¹ K ₂ O	organic ^a 30 kg ha ⁻¹ N 30 kg ha ⁻¹ P ₂ O ₅ 30 kg ha ⁻¹ K ₂ O
weed control	herbicide application postemergence	Plant Protection spring-time harrowing	herbicide application postemergence	spring-time harrowing
pest control	none	none	none	none
special treatments	residues removed	residues incorporated		

^a An estimated additional amount of 70 kg ha⁻¹ N after clover as intercrop. Table is slightly modified from ref 18.

research is needed to evaluate the influence of other factors, such as climate, location, and farming practice.

The objective of this study was to examine the influence of the farming system on the lutein content of soft and hard wheat under controlled conditions. For this, samples derived from long-term field experiments were analyzed. Furthermore, soft wheat samples from farm pairs (same variety and location) were analyzed to test the degree to which the results from controlled conditions (field trials) can be extended to a broader variability of cultivation processes and locations.

MATERIALS AND METHODS

Chemicals. Solvents for extraction and HPLC (methanol, acetonitrile, tetrahydrofuran, 2-propanol) were of gradient grade (provided by VWR Prolabo, Hannover, Germany). Lutein (Applichem, Darmstadt, Germany), zeaxanthin (Roth Laborchemie, Karlsruhe, Germany), butylated hydroxytoluene (Merck, Darmstadt, Germany), and 8'-β-apo-carotenol (Sigma-Aldrich, Munich, Germany) were of the highest commercially available grade. The water used for the analyses was prepared by a Milli-Q Gradient A10 TOC facility (Millipore, Schwabach, Germany).

Wheat Samples. Samples come from two long-term field experiments comparing organic and conventional production systems. First, soft wheat of the DOC trial (dynamic-organic-conventional) of the years 2005 and 2006 was analyzed. In the DOC trial farming systems differing in plant protection management and fertilization were compared (for a comparison of all variants see **Table 1**). A more comprehensive description of the trial is given in ref 17. From 2005 to 2006 the varieties of the produced wheat changed from 'Titlis' to 'Runal'. Both varieties are typical for Swiss agriculture.

Second, hard and soft wheats of the MASCOT experiment (Mediterranean arable system comparison trial) of the years 2005 and 2006

were analyzed. A conventional and an organic variant, both not irrigated for the wheat sites, were compared. The conventional variant is characterized by a moderate to high use of agrochemicals and a higher impact of mineral fertilizers (see **Table 2**). A more detailed description of the experimental design is given in ref 17.

Additionally, three soft wheat samples were examined deriving from farm pairs producing conventionally and organic, respectively. The only common feature of the organic farms was production according to EU Regulation 2092/91. No further regulation of the cultivation properties was set up for this experiment for organic or conventional produce, respectively. The farm pairs were located in different German regions (see **Table 3**). Each farm pair consisted of two farms with a small distance between (<10 km). One of the farm pairs cultivated the variety 'Ludwig'; the other two cultivated the variety 'Capo'. Both are commonly used varieties in organic farming in Germany.

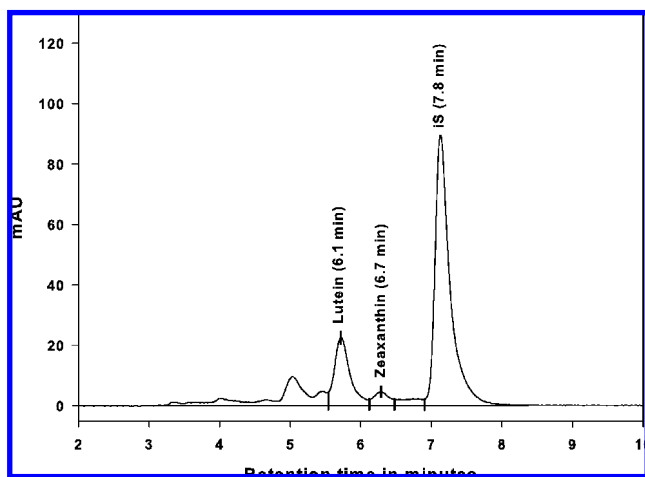
A minimum of 800 g of wheat from each variant was analyzed. The wheat samples were cleaned manually to remove impurities such as glumes, weed seeds, and dirt. The samples were stored in paper bags under laboratory conditions. All samples were given coded to the laboratory. Decoding was done after transmission of the results.

HPLC Analysis of the Xanthophylls. For the xanthophyll analysis an aliquot of 80 g was milled in a centrifugal mill for laboratory use (ZM-100, provided by Retsch, Haan, Germany). Four replicates were analyzed as follows: 2 g of the flour and 200 mg of magnesium hydroxide carbonate were weighed into 50 mL centrifugal tubes. Two hundred microliters of an 80 μg mL⁻¹ solution of 8'-β-apo-carotenol as internal standard was added. Then the flour was mixed with 4 mL of HPLC water using a glass stirrer. The stirrer remained in the sample until the end of extraction. The flour was allowed to swell for 10 min. After that 10 mL of a mixture of tetrahydrofuran and methanol was added and intensely mixed. The extraction was performed in an ultrasonic bath at <10 °C for 15 min. The samples were centrifuged for 10 min at 4 °C. The supernatant was collected in another centrifugal

Table 3. Variety and Location for the Farm Pair Samples

variety	abbreviation ^a	cultivation	location (all in Germany)	latitude and longitude	distance in km
Ludwig	CON/A	conventional	31180 Ahrbergen (K)	52° 13' N, 09° 52' E	7
	ORG/A	organic	31191 Algermissen	52° 14' N, 10° 3' E	
Capo	CON/B	conventional	97490 Poppenhausen	50° 5' N, 10° 8' E	7
	ORG/B	organic	97729 Ramstal	50° 8' N, 10° 4' E	
	CON/C	conventional	16278 Wilmersdorf	53° 6' N, 13° 55' E	<2
	ORG/C	organic	16278 Wilmersdorf	53° 6' N, 13° 55' E	

^a Samples with the same letter followed by the "r" according to one farm pair.

**Figure 1.** Chromatogram of a soft wheat sample.**Table 4.** Spectral Data for the Detected Compounds As Measured under the Described Chromatographic Conditions

compound	maxima ^a (λ , nm)	% III/II ^b
lutein	424; 445 ; 472	66
zeaxanthin	426; 450 ; 478	38
internal standard	462	

^a The maximum with the highest absorption is printed bold. ^b Numerical expression of the structure of the spectra. The value is a quotient of the height of the two highest maxima. It is calculated according to ref 29.

tube. The extraction of the flour was performed three times, and after that the supernatant was colorless.

Ten milliliters of 10% NaCl in water was added to the collected supernatants. This solution was mixed with *n*-hexane containing 0.1% butylated hydroxytoluene as antioxidant. The upper phase containing the xanthophylls was transferred to a flask. The remaining solution was reextracted twice.

The collected *n*-hexane phases were evaporated in a rotary evaporator until dryness at reduced pressure. The residue was redissolved with 0.4 mL of tetrahydrofuran/methanol (1:1, v/v) and 0.4 mL of the mobile phase. The solution was filtered over a 0.45 μ m syringe filter and filled into a brown glass vial. It was analyzed immediately by HPLC. The whole extraction process was performed at reduced light.

The HPLC was performed on a Waters Alliance 2695 equipped with a 2996 diode array detector. Fifteen microliters of the sample eluted isocratically at 1 mL min⁻¹ with a mixture of methanol, acetonitrile, and 2-propanol (54:44:2, v/v/v) was used to separate the xanthophylls. The separation was performed on a Grace-Vydac 201TP54 reversed-phase (4.6 mm \times 250 mm) polymeric C18 column with a guard column containing the same stationary phase at 25 °C. The sample run time was 15 min including the time for column cleanup and reequilibration. The xanthophylls were detected at 450 nm wavelength. The identification was done by comparison of the spectra and retention times to synthetic standards (see **Figure 1** and **Table 4**). Quantification was done by using the peak height; calibration was done with the synthetic standard.

Dry Matter and Thousand-Kernel Weight (TKW). For the determination of the dry matter an aliquot of the flour used for the

Table 5. Lutein and Zeaxanthin Concentrations of the DOC Trial Samples ($N = 16$ per Treatment)

sample ^a	lutein ^a (μ g g ⁻¹ of dry matter, mean \pm SD)	zeaxanthin ^a (μ g g ⁻¹ of dry matter, mean \pm SD)
Year 2005, Variety 'Titlis'		
NOFERT	2.2 \pm 0.18	0.23 \pm 0.010
BIODYN	1.7 \pm 0.14	0.20 \pm 0.030
BIOORG	1.7 \pm 0.10	0.20 \pm 0.015
BIODYN + BIOORG	1.7 \pm 0.12	0.20 \pm 0.023
CONFYM	1.6 \pm 0.08	0.18 \pm 0.013
CONMIN	1.6 \pm 0.14	0.18 \pm 0.018
CONMIN + CONFYM	1.6 \pm 0.11	0.18 \pm 0.015
Year 2006, Variety 'Runal'		
NOFERT	1.8 \pm 0.05	0.34 \pm 0.018
BIODYN	1.5 \pm 0.12	0.29 \pm 0.016
BIOORG	1.6 \pm 0.07	0.31 \pm 0.025
BIODYN + BIOORG	1.6 \pm 0.10	0.30 \pm 0.024
CONFYM	1.4 \pm 0.04	0.31 \pm 0.022
CONMIN	1.4 \pm 0.15	0.32 \pm 0.030
CONMIN + CONFYM	1.3 \pm 0.11	0.32 \pm 0.026

^a Additionally, the aggregated conventional and organic variants were compared (see the additional rows for "organic variants" and "conventional variants").

xanthophyll analysis was used. Three replicates were dried for 24 h at 105 °C. For statistical analysis the mean of the three determinations was used.

About 200 g of each sample was analyzed for the TKW. Therefore, 1000 kernels were counted with a seed counter and weighed on a laboratory balance. The determination was repeated with three replicates per sample. For statistical analysis the mean of the three determinations was used.

Statistical Analysis. Statistical analysis was performed using SPSS 14.0. Data were analyzed by ANOVA and following the Tamhane test for comparisons of multiple means. Due to the experimental design of the MASOCOT experiment only pairwise tests for each block were performed.

RESULTS AND DISCUSSION

DOC Trial Samples. As shown in **Table 1** there were only small differences of the lutein and zeaxanthin concentrations between the fertilized variants (**Table 5**). The lutein concentrations of the two varieties/years were comparable. In contrast, the zeaxanthin concentrations of the 'Runal' variety/year 2006 were 1.5-fold higher than for the 'Titlis' variety/year 2005. There were no significant differences of the lutein concentrations within the organic and the conventional variants, respectively. For the zeaxanthin content there was only a significant difference of the BIODYN and the BIOORG variant for the 'Runal' variety. Even if the number of variants is reduced from five to three, there were significant differences only for the lutein concentration of 'Titlis'. The lutein concentration of the organically produced wheat tends to be higher than the concentration of the conventional produce. The zeaxanthin concentration differed significantly for both varieties, but the differences had opposite signs for the two varieties (see **Figure 2**).

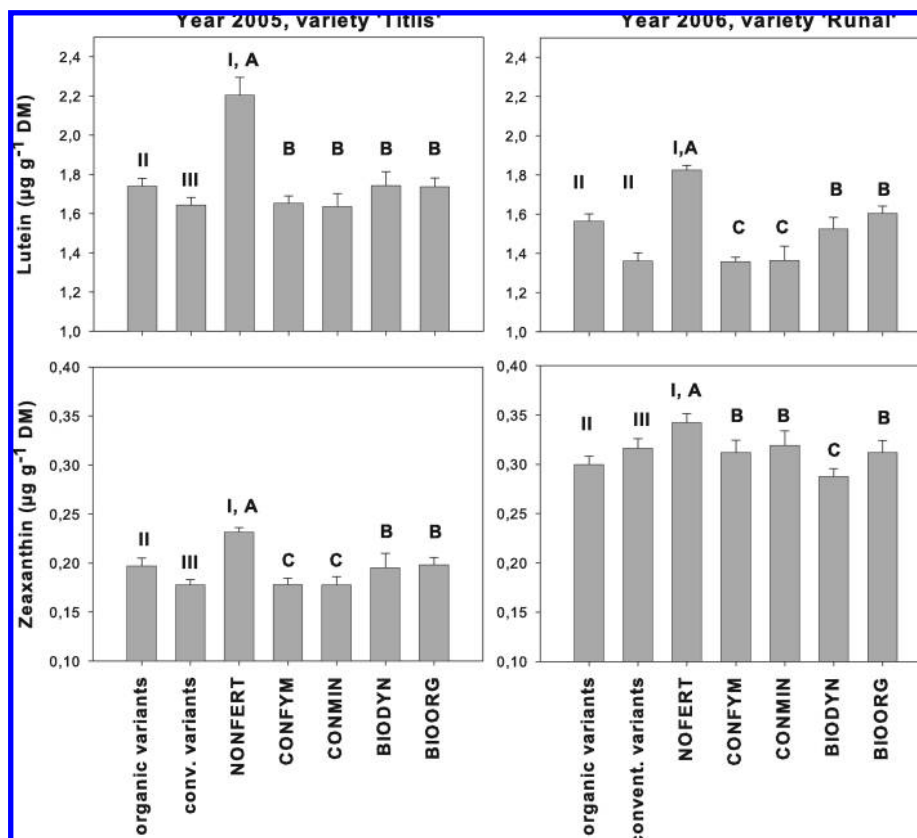


Figure 2. Lutein and zeaxanthin concentrations in dry matter of the DOC trial samples. See Table 5 for description of “organic variants” and “conventional variants”.

Table 6. Lutein and Zeaxanthin Concentrations per Block of the MASCOT Wheat Samples ($N = 4$ per Variant)

sample	soft wheat		hard wheat	
	lutein ($\mu\text{g g}^{-1}$ of DM, mean \pm SD)	zeaxanthin ($\mu\text{g g}^{-1}$ of DM, mean \pm SD)	lutein ($\mu\text{g g}^{-1}$ of DM, mean \pm SD)	zeaxanthin ($\mu\text{g g}^{-1}$ of DM, mean \pm SD)
	Year 2005			
block 1, org	1.2 \pm 0.01	0.19 \pm 0.008	3.9 \pm 0.22	0.28 \pm 0.021
block 1, conv	1.1 \pm 0.04	0.25 \pm 0.007	4.0 \pm 0.09	0.27 \pm 0.011
difference ^a	8.3	-31.5		
<i>p</i> value	<0.001	<0.001	0.801	0.204
block 2, org	1.2 \pm 0.01	0.24 \pm 0.011	3.7 \pm 0.04	0.21 \pm 0.007
block 2, conv	1.2 \pm 0.02	0.21 \pm 0.007	4.1 \pm 0.09	0.32 \pm 0.005
difference ^a		12.5	-10.8	-52.4
<i>p</i> value	0.094	0.024	<0.001	<0.001
block 3, org	1.3 \pm 0.03	0.26 \pm 0.010	3.8 \pm 0.09	0.26 \pm 0.008
block 3, conv	1.2 \pm 0.03	0.26 \pm 0.014	3.6 \pm 0.06	0.30 \pm 0.003
difference ^a	7.7		5.0	-15.4
<i>p</i> value	0.011	0.909	0.015	<0.001
	Year 2006			
block 1, org	1.2 \pm 0.02	0.23 \pm 0.003	3.1 \pm 0.08	0.28 \pm 0.16
block 1, conv	1.2 \pm 0.04	0.28 \pm 0.013	3.9 \pm 0.17	0.38 \pm 0.03
difference ^a		-21.7	-25.8	-35.7
<i>p</i> value	0.835	0.001	<0.001	0.002
block 2, org	1.4 \pm 0.02	0.27 \pm 0.018	3.3 \pm 0.14	0.31 \pm 0.029
block 2, conv	1.2 \pm 0.01	0.26 \pm 0.015	3.2 \pm 0.04	0.29 \pm 0.03
difference ^a	14.3			
<i>p</i> value	<0.001	0.310	0.175	0.830
block 3, org	1.3 \pm 0.03	0.29 \pm 0.023	3.2 \pm 0.03	0.30 \pm 0.021
block 3, conv	1.2 \pm 0.02	0.27 \pm 0.023	4.4 \pm 0.09	0.40 \pm 0.051
difference ^a	7.7		-27.3	-33.3
<i>p</i> value	<0.001	0.489	<0.001	0.012

^a The difference is given only when significant (indicated by bold type). It is expressed in percent of the organic variant. The given *p* value is calculated by ANOVA.

MASCOT Samples. Within the MASCOT experiment two wheat species (*Triticum aestivum* L. and *Triticum durum* L.) were compared. Because the experiment is designed as a block trial, the variants (organic vs conventional) were compared for each block separately (see remarks according to the experimental design

in ref 18, p 6). Therefore, the block is interpreted as repeated measurement.

For the soft wheat there were significant differences of the concentrations of both xanthophylls. These differences were not significant for all blocks. The differences between the blocks

Table 7. Lutein and Zeaxanthin Concentrations per Block of the Farm Pair Samples ($N = 4$ per Variant)

sample ^a	lutein ($\mu\text{g g}^{-1}$ of DM, mean \pm SD)	zeaxanthin ($\mu\text{g g}^{-1}$ of DM, mean \pm SD)
Variety 'Ludwig'		
CON/A	1.5 \pm 0.05	0.27 \pm 0.011
ORG/A	1.5 \pm 0.09	0.31 \pm 0.017
difference ^b		12.9
<i>p</i> value	0.411	0.021
Variety 'Capo'		
CON/B	1.0 \pm 0.01	0.17 \pm 0.002
ORG/B	1.0 \pm 0.03	0.20 \pm 0.006
difference ^b	<5.0	15.0
<i>p</i> value	0.024	<0.001
CON/C	1.3 \pm 0.04	0.14 \pm 0.008
ORG/C	1.3 \pm 0.03	0.13 \pm 0.008
difference ^b		
<i>p</i> value	0.476	0.075

^a Abbreviations: see **Table 3**; ^b The difference (organic minus conventional) is given in percent of the concentration of the organic variant. The difference is given only when significant. The given *p* value is calculated by ANOVA.

and the variants were comparable and small ($\Delta c_{\text{lutein}} = 0.1\text{--}0.2 \mu\text{g g}^{-1}$). As shown for the DOC samples the lutein concentration of the organically produced wheat tends to be higher than the concentration of the conventional produce. For the zeaxanthin there was no unique influence of the farming system (**Table 6**, columns 2 and 3).

As for the soft wheat there were no significant differences of the concentrations of the xanthophylls for the hard wheat throughout the blocks. In the first year there was no unique influence of the farming system on the lutein concentration, whereas in the second year the concentration of the organic produce was significantly lower for two of the three blocks. The opposite was found when the soft wheat was measured. The zeaxanthin concentration was lower in the organic samples in both years but not for all blocks, too (**Table 6**, columns 4 and 5).

Farm Pair Samples. For the analysis of variance within the cultivation methods samples of three farm pairs were analyzed. As for the field trial samples there were only small differences between the variants of each farm pair. No significant difference could be found for either lutein or zeaxanthin. For two of the

three farm samples the zeaxanthin concentration of the organic variant was higher, whereas the lutein content was equal compared with the conventional variant (**Table 7**).

Correlation of Lutein Content and Thousand-Kernel Weight. As denoted, the xanthophylls are located mainly in the aleuron layer of the wheat kernels. Therefore, the xanthophyll concentration may depend on the quotient of the kernel surface area to the kernel weight. The kernel weight can be substituted by the TKW.

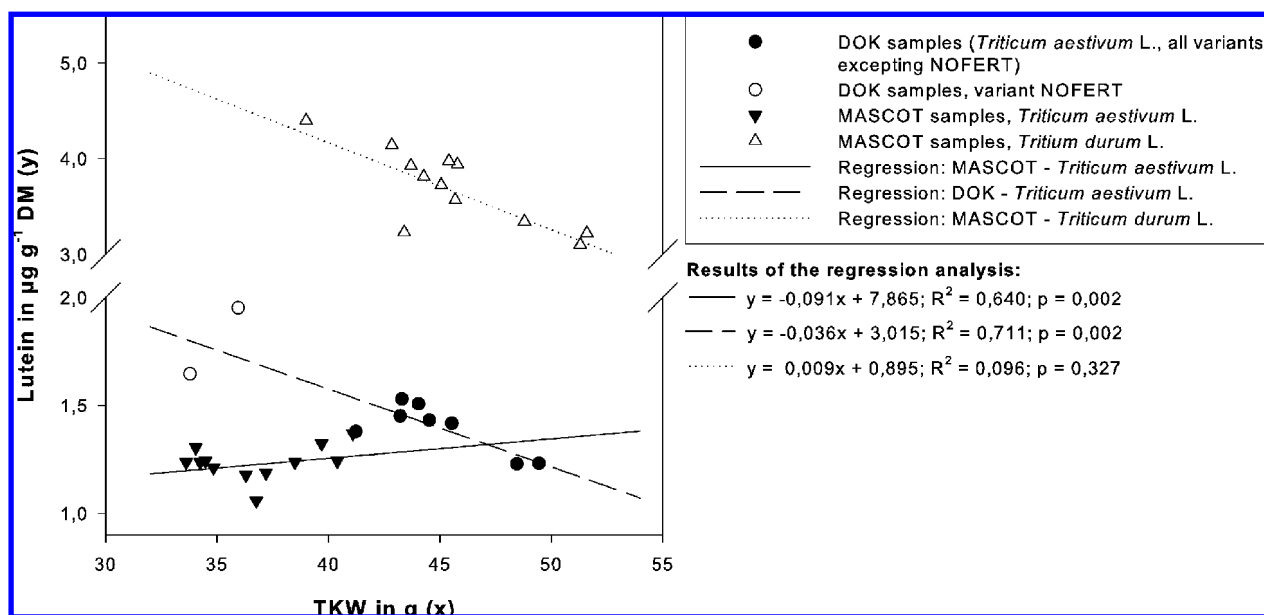
For statistical analysis of the correlation of the TKW and the lutein concentration only the DOC and the MASCOT samples were used. There is a negative correlation for the DOC samples and the *T. durum* L. samples of the MASCOT experiment (**Figure 3**). For zeaxanthin there was no significant correlation at all.

Lutein concentrations were found in a wide range (1.0–4.4 $\mu\text{g g}^{-1}$ of DM). This fits with the data given in the literature for soft and hard wheat (3, 5, 10, 22–24). The lutein concentrations found for soft wheat are rather high in comparison to the data given in the literature. This may be the result of the use of an optimized extraction method (26). Additional zeaxanthin was found in all samples. The zeaxanthin concentration varied within a range of 0.08–0.40 $\mu\text{g g}^{-1}$ of DM, which fits the data of Adom (5) (range = 0.09–0.32 $\mu\text{g g}^{-1}$ of DM).

So far, only the genetic variability of the xanthophyll content of wheat has been examined. Only in one paper was the influence of the location on the lutein content shown (25). With this study the influence of the farming system on the xanthophyll concentration was examined.

Only a small influence of the farming system on the xanthophyll content was examined. For lutein, a tendency of higher concentrations (soft wheat) or lower concentrations (hard wheat) in organic produce was found for the long-term field trial samples. For the farm pair samples no significant differences were found. The zeaxanthin concentration was less influenced by the farming system than the lutein content.

Even though there is no significant difference, there is a trend for higher lutein concentrations of the organically produced soft wheat. Lutein is mainly concentrated in the aleuronic layer of the wheat kernels (6). There is a relationship between the lutein concentration and the surface area of the wheat kernels. The TKW is a function of the volume of the wheat kernels. Because

**Figure 3.** Correlation of the TKW (x-axis) and the lutein concentration (y-axis) for the DOC and the MASCOT samples.

of the mathematical relationship of surface area and volume, a negative correlation of TKW and lutein concentration can be estimated. For the field-trial samples this was tested: A negative correlation for the DOC samples and the hard wheat samples of the MASCOT trial was found. The missing significant correlation of the *T. aestivum* L. samples can be explained by very low variance of the lutein contents and TKWs.

The TKW is strongly influenced by N fertilization (27). For the DOC samples this can be shown; the ranking of the TKW is in the order NOFERT > BIODYN = BIOORG > CONMIN = CONFYM (Figure 2; Table 5). This order is consistent with the amount of N fertilization. However, for the hard wheat samples of the MASCOT experiment, this does not fit. Here the higher N fertilization results in lower TKW. According to this, the cause and effect of N fertilization and TKW cannot be reduced by the amount of N fertilization. Rather, the TKW may be influenced by certain factors of cultivation and environment in a complex way. Therefore, mainly the influence of environmental parameters and cultivation method related factors on the lutein content may be related to different TKWs or wheat kernel sizes.

In terms of a plus for healthy nutrition according to the xanthophyll content, organically produced wheat has nearly no advantages or disadvantages compared with conventionally produced wheat. For this purpose the breeding of high-lutein wheat for organic farming systems is definitely the best way (28, 30). Despite the product-related parameters, there are a lot of other advantages of organic agricultural production systems in terms of sustainable agricultural produce [process-related advantages (20)].

ABBREVIATIONS USED

c, concentration; Δc , concentration difference; TKW, thousand-kernel weight; DM, dry matter; SD, standard deviation.

ACKNOWLEDGMENT

We thank the Research Institute of Organic Agriculture FiBL, Frick, Switzerland, Dr. Paul Mäder and the Eidgenössische Forschungsanstalt Agroscope ART, Dr. David Dubois for providing samples of the DOC trial; the University of Pisa, Centro Interdipartimentale di Ricerche Agro-Ambientali "Enrico Avanzi", for promoting samples of the MASCOT trial; the Von Thünen Institute, Prof. Dr. Rahmann, for handling the DOC and farm pair samples; Dipl. Ing. Kirsten Körner for assistance with the literature research; and Gaby Mergardt and Michaela Kunze for assistance in the laboratory.

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Received for review May 6, 2008. Revised manuscript received November 12, 2008. Accepted November 13, 2008. We thank the Federal Ministry of Food, Agriculture and Consumer Protection for funding this study within the Bundesprogramm Ökolandbau (02OE170F).

JF801407V