Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

Organically grown wheat: carotenoid content

Health and socio-economic aspects under organic farming system

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Degree Project • 30 hec
Agroecology - Master´s Programme
Alnarp 2014

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Ekologisk odlat vete: karotenoid halt
Hälso-och socioekonomiska aspekter i ekologisk odling

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Credits: 30 hec
Project Level: A2E
Course Title: Master's Thesis in Agricultural Science/Agroecology
Course Code: EX0486
Programme: Agroecology – master's programme

Place of Publication: Alnarp
Year of Publication: 2014
Cover Art: Eliza Frey
Online Publication: http://stud.epsilon.slu.se

Keywords: Organic wheat, genotypes, carotenoid content, organic farming, life cycle analysis, farmers' perspective.

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Foreword

During the Agroecology program, we have been working on different research projects and practical work. We have been given several lectures about environmental, socio-economic and social sustainability, and resource considerations. It was rewarding in case of new insights into the environment surrounding us, in terms of energy consumption and economic returns of the farms, as well as the possibilities for organic farming as a sustainable method of food production, which includes both consumer health and agricultural sustainability. I was involved in doing projects related to biological plant protection and food crops, other times experiments on organically grown wheat, investigating antioxidants (carotenoids), and assessing the organic wheat production system as a sustainable form of food production.

Due to my previous studies and interest in food science, I was motivated to focus on cereal products to complete the master's thesis. It is useful to relate the past knowledge and benefit of the new information for the future, especially wheat, which is among the major crops in the food market in case of consumer supplies. Thus, the aim of Agroecology and ecological food production could be achieved with relation to cereal crops. The thesis was focused on assessing if organically grown wheat genotypes are a good source of health-related compounds, antioxidants such as carotenoids, in terms of producing higher quality wheat. The project was completed in the form of lab analyses of carotenoid content from organically grown wheat genotypes, together with a literature review study about life cycle analysis (LCA) of organic wheat farming in terms of socio-economic and environmental sustainability compared with conventionally grown wheat. Farmers' perspectives about growing organic wheat were also included, with a focus on health aspects.

In all, one of the main conclusions was that the studied organic wheat genotypes were found to exhibit varying carotenoid profiles consisting of mainly lutein with small amounts of other carotenoids. Selected specific organic wheat genotypes might be promising for promoting human health, and their use would be sustainable, considering the environmental benefits derived from the organic farming system. At the same time the nutritional quality of all the studied material, in general, did not prove to be higher as compared with conventional wheat, with the exception of some specific genotypes.

As a student with agroecology education, I should be able to utilize the knowledge in agricultural sustainability, with the goal of promoting healthier and more sustainable food production. The path could be followed towards extending the knowledge and implementing it practically, with utilization of extension and farming advisory organizations, to assist farmers to develop organic systems. Non Governmental Organizations (NGO), agricultural research institutes and universities could be among the places to work in the future as an agroecologist.

Fatemeh Yousefi, January 2013

Abstract

In today's world, there is more awareness than ever before about the relationship between food consumption and human health. This awareness is especially relevant for cereals, including wheat, which is one of the most important crops in terms of feeding the world. The aim of this study was to evaluate genotypic influence on carotenoid amount in organically grown wheat genotypes. Furthermore, life cycle analyses of organic wheat farming in the scientific literature were reviewed and compared to similar analyses of conventional wheat farming in order to assess socio-economic and environmental sustainability differences between the two systems. A questionnaire was developed and used as a guideline for a literature review on farmers' opinions about organic wheat farming. The experimental phase was done through analyzing amounts of four carotenoids (e.g. β-carotene, β-cryptoxanthin, lutein and zeaxanthin), in 33 organically grown wheat genotypes by high performance liquid chromatography (HPLC). Carotenoid contents were compared between and within four genotypic wheat groups; *landraces*, *spelt*, *primitive* and *old cultivars*, as well as between *winter* and *spring* wheats. Within this study, no statistically significant differences of total carotenoids amounts were found across the four wheat groups and the two wheat types. Among the 33 genotypes, total carotenoid amounts were significantly higher in *Olands 8*, *Schweiz* and *Aura*, 4.08, 2.49 and 2.47 (mg/kg of DW), respectively, than in all other genotypes. These results were compared with reports on conventional wheat, and no significant differences were found in carotenoid amounts across studies. Our results on carotenoid content can be combined with findings from previous studies that examined other antioxidant compounds in these same genotypes. While, organic wheat production could benefit the environment, e.g. less water contamination and improved soil quality, there are also drawbacks to the organic wheat farming system. For example, it was demonstrated that organic farming system is not more environmentally sustainable than the conventional systems if the organic market is farther than 2000 Km from the farm, which results in equal fossil fuel consumption and global warming potential (GWP) in both systems. In conclusion, given that some genotypes were identified with significantly higher amounts of total carotenoids, it could be of great value to use these genotypes in future breeding programs to increase the nutritional value of wheat. Organic wheat production could be sustainable in several aspects, such as improved soil quality and potentially reduced GWP, but with a holistic view, several factors exist that could limit its sustainability. For example, economical considerations are often a greater concern than environmental impacts for both farmers and consumers. To realize the full potential of organic wheat farming, more research should be done to inspire consumers and wheat farmers towards broader thinking about organic wheat production and consumption with consideration of total nutrient content as well as environmental and economic sustainability.

Keywords: Organic wheat, genotypes, carotenoid content, organic farming, life cycle analysis, farmers' perspective.

Abbreviations:

ANOVA - Analysis of Variance
CO_2-eq – Carbon Dioxide Equivalent
DW – Dry Weight
GHG – Green House Gases
GLM – Generalized Linear Model
GMO – Genetically Modified Organisms
GWP – Global Warming Potential
HM – Heavy Metal
HPLC – High Performance Liquid Chromatography
HSD – Honestly Significant Difference
IFOAM **-** International Federation of Organic Agriculture
LCA – Life Cycle Analysis
LSD – Least Significant Difference
N – Nitrogen
N_2O – Nitrous Oxide
NGO – Non Governmental Organizations
SD – Standard Deviation
USDA – US department of Agriculture

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1. Introduction

Conventional farming is the most dominant system of farming nowadays with its main focus to produce high crop yields and increased economical benefits (Pimentel *et al.*, 2005). This farming system includes intensive mechanized farming, as well as the use of pesticides and synthetic fertilizers, which bring environmental costs, such as soil erosion due to nitrogen fertilizers, water contamination, release of greenhouse gases, pest resistance and loss of biodiversity (Pimentel *et al.*, 2005, Wood *et al.*, 2006). In addition, due to pesticide use, some toxic chemicals can be delivered to the food products (Pimentel *et al.*, 2005). Therefore, different methodologies that could contribute towards reducing the negative impacts of conventional farming systems have been explored. One such paradigm that has been given great focus in recent years is the organic farming system (Pimentel *et al.*, 2005).

1.1 Organic agriculture

Organic agriculture consists of both a philosophy and a system of farming aiming for social, environmental and economic sustainability (Edwards-Jones and Howells, 2001). Organic farming aims to promote and enhances biodiversity, biological cycles and soil biological activity (IFOAM, 1998). Furthermore, organic farming is grounded on no use of synthetic chemical inputs and relies on management practices that restore, preserve and improve the ecosystem (Maeder *et al.*, 2002). Specifically, in organic production, chemical fertilizer and pesticide inputs are replaced with organic fertilizers and pesticides, which results in production of foods free of synthetic chemicals. One concern resulting from the lack of synthetic chemical inputs in organic agriculture relates to contamination by fungal toxins such as mycotoxins. Since synthetic fungicides would not be used in the organic farming system, it has been suggested that there is an increase risk of fungal contamination in both agricultural crops and post-harvest stored products. However, while there are reports of contamination of organic products with fungal toxins, it has not been conclusively demonstrated that contamination of organic farming products by fungal toxins occurs at a significantly greater level than for conventional farming products (Lairon, 2011).

The organic production area in Sweden is about 392 thousand hectares (FiBl and IFOAM, 2011) and during recent years, there has been an increase in the organic production area in Sweden and U.S. which results in an increase in consumption of organic food (FiBl and IFOAM, 2011, Schaak *et al.*, 2011). Cereals including wheat are among the main crops grown organically in Sweden (SCB, 2009).

1.2 Conventional farming versus organic farming

In transitioning from conventional to organic production, the focus on obtaining high yields is shifted towards higher quality products in organic systems (Tamis and van den Brink, 1999). There is also a shift towards the development of more integrated and ecologically-friendly farming practices (Jensen *et al.*, 2011).

Organic farming is accomplished by lower fossil fuel consumption and avoiding toxic chemicals (synthetic pesticides) as well as, avoiding practices that are harmful to soil ecosystems, like excessive tillage (Maeder *et al.*, 2002, Reganold *et al.*, 2001). Also, in organic farming a common problem e.g. low yield, can be compensated by higher prices for the organic products (Tamis and van den Brink, 1999). Furthermore, consumers' demands are increasing towards healthier diets, including natural or organic foods and cereals. Organically produced food with no synthetic chemical inputs is considered to be higher quality food as compared with conventional food (Badgley *et al.*, 2007). However, organic production, due to the limited yield and difficulties of organic N inputs, is not reasonable to implement in every part of the world especially in highly populated countries, where food supply for the growing population is given more importance than higher nutritional products. Also, farmers' perspectives can vary in different parts of the world, due to economic concerns, as well as knowledge and support regarding organic farming (Chizari *et al.*, 2001).

1.3 Wheat

Wheat is one of the world's major food crops. Thus, the nutritional value of wheat is of great importance, as it contributes significantly to the human diet in terms of calories and nutrients (Abdel-Aal *et al.*, 1998). In modern agriculture, bread wheat (*T. aestivum*) is widely grown all over the world, accounting for 95% of total wheat grown. The remaining 5% largely consists of durum wheat (*T. durum*) (Shewry, 2009). Currently, a total of around 4000 bread wheat varieties are cultivated in the world, with either a spring or winter growth habit (Posner, 2000). It has been suggested that modern cultivated varieties of bread wheat that are conventionally grown are not suitable for organic farming, because they lack important characteristics that facilitate successful application of organic farming, such as more efficient root systems, high competitiveness with weeds, and high resistance to common diseases (Konvalina *et al.*, 2009).

1.3.1. Wheat groups

Spelt wheat (*Triticum spelta L.*) is considered a sub species of common bread wheat that was widely used in the past, but its production in agricultural systems is limited today to a much smaller economic scale (Bonafaccia *et al.*, 2000). In some parts of Europe, spelt wheat is organically cultivated as a result of several qualities that make it suitable for sustainable farming (Moudrý and Dvořáček, 1999). For example, spelt wheat needs less nutrient inputs, i.e. fertilizers, due to an efficient root system (Vasil and Vasil, 1999, Ehsanzadeh, 1999). Spelt wheat plants are taller compared with bread wheats and are able to outcompete weeds (Konvalina *et al.*, 2011). Furthermore, spelt wheats have also been shown to contain higher yields of protein amounts (Kg per ha) and protein content (as a percentage of overall material content), as well as a higher percentage of wet gluten and lower gluten index than modern wheat varieties (Konvalina *et al.*, 2012).

Primitive wheats are the earliest cultivated form of wheat with ancient origins (Hammer, 2000). Primitive varieties such as Einkorn (*Triticum monococcum*) and Emmer (*T. dicoccum*) have been found to have high nutritional value with respect to specific properties. For example, *Einkorn* has a high level of carotenoids and high protein concentration, compared to *modern* wheat (Grausgruber *et al.*, 2004). *Primitive* wheats also have properties suitable for organic farming and might be an optimal genotype for producing high nutritional value food (Hussain *et al.*, 2010).

In this thesis, similar terminology to group the wheat genotypes has been adopted as was previously used by Hussain (2012). Thus, *old cultivars* refer to varieties cultivated during the first half of 20th century (Hussain, 2012), as distinguished from those varieties that have been released since the 1970's. Based on previous studies, *old cultivars* generally contain higher mineral contents than *modern cultivars* (Hussain *et al.,* 2010). Other authors point out that *old cultivars* have higher grain protein concentrations (Gooding *et al.*, 1999), and have better drought resistant properties (Dencic *et al.*, 2000).

Landrace cultivars refer to wheat varieties that are grown in distinct regions adapted to local climates. From one region to another, *landrace* varieties can have dramatically different traits, thus, in considering *landrace* varieties worldwide, a potential source of genetic diversity exists (Zeven, 1998). These qualities can be exploited to develop specialised breeds adapted to the demands of organic farming systems. For example, appropriately selected, naturally taller, *landrace* wheat varieties are more likely to be farmed successfully by organic methods, because they are able to outcompete weeds better (Konvalina *et al.*, 2011). Other traits such as disease resistance, nutrient content and colour have value for producers and consumers and could be selected from different *landrace* varieties.

1.3.2. Nutritional value of organic wheat

It has been reported that organically grown wheat is nutritionally equivalent to conventionally produced wheat (Langenkamper *et al., 2006).* Differences in nutritional composition of wheat may arise due to the amount of fertilizers used, growing conditions, environment, cultivars, etc. (Dangour *et al.*, 2009). Organic and conventional farming have been shown to have a large influence on the concentrations of minerals and other healthy compounds in wheat. A study from Hussain *et al.* (2010) showed that high mineral concentrations, close to recommended daily requirements, can be obtained by organically grown specific *primitive* wheat genotypes.

In the industrialized world where increased amounts of free radicals could cause a greater degree of chronic diseases, including cancer and heart problems, antioxidants in wheat products can help protect the body from these chronic disorders, and are thus thought of as anti-disease and anti-aging nutrients (Rodriguez-Amaya, 2010). Antioxidants also enhance the body's immune system, by providing a defense to the body against free radicals (Jacob, 1995). A combination of several antioxidants works better than single antioxidant to enhance the body's defense against free radicals (Jacob, 1995).

1.3.3. Carotenoids

Carotenoids are an important class of compounds that function as both pro-vitamins and antioxidants in the body. Humans and animals cannot synthesize carotenoids, but obtain them from their diets, which include carotenoid-containing foods (Zile, 1998).

Typically 50 to 60 carotenoids exist in the human diet, enriched in green, yellow/red, or yellow/orange vegetables and grains, including wheat (Dembinska-Kiec, 2005). The most abundant carotenoids are β-carotene, lycopene, lutein, cryptoxanthin, α-carotene and zeaxanthin. In wheat, lutein is well known to be the most abundant carotenoid, comprising 70 to 80% of total carotenoid content (Abdel-Aal *et al.,* 2007, Konopka *et al.*, 2006, Lepage and Sims, 1968). Other carotenoids commonly found in wheat include lutein esters and also β-carotene, β-cryptoxanthin and zeaxanthin (Abdel-Aal *et al.*, 2007). Potential health benefits of dietary carotenoids have been identified. β-cryptoxanthin and zeaxanthin, may help protect individuals from chronic diseases including cancer by decreasing oxidative damage (Leenhardt *et al.*, 2006). In a study by (Fernandez-Garcia *et al.*, 2012) it was demonstrated that β-carotene consumption reduces the incidence of some types of cancer. β-carotene is also the key source of pro-vitamin A (Dembinska-Kiec, 2005).

1.3.4. Effect of genotype and environment on wheat nutritional value

It is known that genetic and environmental factors influence wheat quality (Hussain *et al.*, 2009). In previous studies on organic wheat by Hussain *et al.* (2012a) it was found that high amounts of health positive compounds i.e. *tochopherols* and *tocotrienols*, were found in specific wheat genotypes (Odin, Jacoby 59 utan borst and Olympia). In wheat, the nutrient composition is influenced by the cultivar, maturity at harvest, climate, season and soil conditions. Furthermore, geographic site of production, part of the plant utilized, farming practices, harvesting and post-harvest handling, processing and storage conditions play an important role (Rodriguez-Amaya, 2010).

1.4 Life Cycle Analysis (LCA)

In order to evaluate different aspects of organic wheat farming as a stand-alone agricultural system as well as compared to conventional farming, it is necessary to thoroughly analyze the benefits and drawbacks of the two systems at every level. This can be done using life cycle analysis (LCA), which consists of the assessment of economical, environmental and social sustainability of the two systems. This includes obtaining farmers'/consumers' perspective on the effects of the different systems on their health and quality of the organic wheat. Farmers are one of the most significant input factors to run agricultural systems more sustainably. So, it's essential to analyze farmers' ideas to obtain a systematic perspective on organic farming.

1.5 Thesis rationale

Wheat is among the major crops feeding the population. Due to intensive modern agriculture using conventional methods, chemical fertilizers and pesticides applied to the soil and overall ecosystem can have damaging effects on the environment as well as on human health. This has led to consideration of complementary farming systems that are more sustainable in terms of the environment and human health, while still being economically viable. Specifically, organic farming systems have been an area of intense focus in recent years. Additionally, from consumers' point of view, there is a desire to have a health positive effect from food. Cereals, such as wheat, can positively contribute to human health due to amounts of proteins, minerals, vitamins and anti-oxidants. In previous studies, older wheat genotypes, which have been grown organically, showed high amounts of specific minerals (zinc, iron etc.) and antioxidants (e.g. tocopherols, tocotrienols) (Hussain *et al.*, 2012a). Therefore, I had an interest to investigate other health compounds e.g. carotenoids. Dietary carotenoids are compounds that can reduce the risk of cancer and cardiovascular diseases and positively contribute to health. In this project, we aimed to investigate carotenoid contents in organically grown wheat from diverse genotypic origins, other than common bread wheat varieties. Based on the results of present and previous studies, it is possible to incorporate our findings into plant breeding programs with expected outcome of increasing the nutritional quality of wheat products. In addition, LCA is a standard systematic methodology that can be used to analyse sustainability of e.g. agricultural systems. In order to assess sustainable viability of organic wheat farming as compared to conventional wheat farming, it is desirable to review LCA studies of these different farming systems.

1.6 Aims and hypothesis

The overall aim of this thesis was to study the carotenoid composition of organically grown wheat genotypes in order to evaluate the specific genotype influence on carotenoid amount. Further, this thesis aimed to evaluate the sustainability of organic wheat production as compared to conventional wheat production, in terms of environmental, economic and social parameters.

The specific aims of this study were to:

- Evaluate the quantity of nutritional compounds, specifically carotenoids, from genetically diverse organically grown wheat. This was done by analyzing the organically grown wheat from different genotypic groups, and evaluating whether they are a good source of health promoting substances, carotenoids. Assessment included, analyzing carotenoid amount in 33 organically grown wheat genotypes, within and between four groups of wheat e.g. *landraces*, *primitive*, *spelt* and *old cultivars*, and winter and spring wheats.
- Summarize life cycle analysis (LCA) of organic wheat through literature review, including economic (energy use and profitability), environmental and social sustainability (farmers' and consumer's preference). Also, the outcomes from the LCA of organic wheat farming were compared with the conventional wheat farming system. No aspect of

LCA was performed experimentally. All conclusions from LCA were drawn from a literature review.

- Develop a questionnaire for evaluating farmers' preferences on growing/ consuming organic wheat with potentially higher amounts of nutritional compounds, including overall contribution to environment and economy. This questionnaire was not distributed as a case study in this thesis, but instead would form the basis for a future investigation. However, elements of the questionnaire were addressed through literature review of farmers' surveys based on similar questionnaires.

It is hypothesized first that older wheat genotypes of organically grown wheat contain greater amounts of carotenoids than modern wheat varieties of organic wheat.

It is also hypothesized that organic wheat farming is more sustainable than conventional wheat farming on regional and global scales. Regarding farmers' opinions and motivations on growing organic wheat, it is hypothesized that different ideologies exist and these will influence their decisions to grow or not to grow organically.

The results of this thesis validate the first hypothesis for some of the organically grown wheat genotypes examined in this study. It will be of interest to investigate these genotypes further as potential targets for wheat breeding programs aimed at increasing the nutritional value of wheat products. However, in general, it was observed that the older organically grown wheat genotypes investigated in this study did not contain significantly different amounts of carotenoids as compared to conventionally grown modern bread varieties. Furthermore, based upon the findings of the literature review based LCA, it has been concluded that while organically grown wheat can be more sustainable, under certain conditions, than conventional in terms of environmental parameters, economic sustainability has not been fully realised.

2. Materials and methods

A total of 33 organically grown wheat genotypes were selected for the study of carotenoids from Hussain *et al.*, (2012a). The material consisted of diverse genotype groups e.g. landraces, old cultivars, spelt and primitive wheat. The grains were stored at 8° C prior analysis. Samples were freeze-dried before analyzing, and were weighted, and milled in the darkness in presence of green light a day prior to analysis and stored in the freezer. Milling of the whole wheat grain into flour was done by a laboratory mill (Yellow line, A10, IKA-Werke, Staufen, Germany).

2.1 Chemicals and equipment

Standards for β–carotene and lutein were purchased from Sigma Aldrich (Buchs, Switzerland), while β-cryptoxanthin and zeaxanthin were purchased from CaroteNature GmbH (Lupsingen, Switzerland). The standard stock solutions of each of the carotenoids were prepared in ethanol working solution and stored at -20° C. High performance liquid chromatography (HPLC) using normal phase column was performed with Waters Empower *3* system.

2.2 Sample preparation

The extraction of carotenoids was performed using the saponification method as in Hussain *et al* (2012a). In this study, 1g of wheat flour was accurately weighted into a screw cap Teflon tube and 2.5 ml of ethanol pyrogallol (60 g/L), 1mL sodium chloride (10 g/L), 1mL ethanol (95%) and 1mL potassium hydroxide (600 g/L) were added. The tubes were capped and transferred to a water bath at 70 ̊ C for 30 min, and were mixed after every 10 min using a vortex-mixer. After saponification the tubes were cooled in an ice-water bath and 7.5 mL of sodium chloride and n-hexane/ethyl acetate (9:1) was added. The blend was centrifuged at a speed of 1500 rpm for 5 minutes and the organic layer was collected for analysis. After the first extraction, additionally two more extractions of the suspension were carried out using 5 mL n-hexane/ ethyl acetate and the organic layer was collected. The organic layer was evaporated to dryness under nitrogen and the dry residue was dissolved in 2mL of n-hexane. Two or three replicates for each sample were prepared. A sample volume of 100 µL was injected for chromatographic analysis. For the recovery procedure 1 g of sample was mixed with known amount of each solution of β–carotene, lutein, β–cryptoxanthin and zeaxanthin.

2.3 HPLC analysis

The separation of carotenoid compounds was achieved using a normal phase HPLC method described by Panfili *et al.*, (2004) with modifications. The mobile phase was n-hexane/isopropyle alcohol and the flow rate was 1.5 mL/min. The wavelength of diode-array was set in the range of 375-500 nm and peaks were quantified at 450 nm. Injection volume was 100 µl/ injection. To verify the precision of the method, repeated calibrations with standard solutions were performed during the analysis of a batch of samples. Results provided a successful determination of carotenoids amounts within an acceptable precision range. Calibration curves were obtained by plotting the peak area of β-carotene, β-cryptoxanthin, lutein, and zeaxanthin versus the concentration of each compound. The percentage of recovery of β–carotene, lutein, β–cryptoxanthin and zeaxanthin standards added to flour sample was determined to obtain the level of carotenoids for analysis and to ensure the accuracy of the method. Percentage recovery for β–carotene, lutein, β–cryptoxanthin and zeaxanthin were 92.6%, 96.6%, 80.4%, 66.1% respectively.

2.4 Statistical analysis

Data were reported as mean amount of carotenoid ± SD (standard deviation). Statistical analysis of the data was performed using SAS statistical software. For evaluating significant differences among mean carotenoid amounts of individual carotenoids across wheat groups, significant differences were determined with one-way analysis of variance (ANOVA) for independent samples with weighted-means analysis, in conjunction with Tukey's Honestly Significant Difference (HSD) test. For evaluating significant differences among mean carotenoid amounts of individual carotenoids across wheat types, Two-tailed student's T-test, with equal variance, was performed. Significant differences were reported when p-value was less than 0.05. For comparison of carotenoids amounts across all individual genotypes, the GLM procedure was performed, and analysis of variance tables were presented. Pairwise comparisons of individual carotenoids across all 33 genotypes were evaluated with Fisher's Least Significant Difference (LSD) test. To demonstrate the significant differences in the amount of individual carotenoids, mean carotenoid amount of each individual carotenoid was compared to mean carotenoid amount of every other individual carotenoid and total carotenoid amounts. For this, LSD was determined and significant differences were indicated where mean minus LSD was greater than LSD and p-value was less than 0.05.

2.5 Questionnaire on farmers' perspectives

A questionnaire was developed for interviews with organic farmers that might be used later on to identify strengths and weaknesses of organic farming systems based on their experiences (Appendix 1). The questionnaire was not sent to farmers as a part of this thesis, due to time constraints. The idea behind the questionnaire was that it could contribute valuable information about organic farming systems, and the questionnaire might be provided via extension services to conventional farmers who might be interested in organic farming. Target areas of the questionnaire include motivations for choosing organic farming, farmers' opinions on their agricultural products, and practical aspects of organic farming. Furthermore, based upon the research question of this thesis, it would be desirable to know if organic farmers would be interested in utilizing genotypes identified to have higher health promoting compounds (e.g. carotenoids).

In the event that the questionnaire study would be performed, the questionnaire should initially be sent to the farmers. A reasonable number of respondents for such an investigation should be 25 initially, based on other studies of this nature (Darnhofer *et al.*, 2005). For those who agree to participate, a semi-structured, face-to-face interview on the farm based on the questionnaire could be scheduled. The interview should be recorded by hand note taking and by tape recorder with the farmer's permission. For those who decline to participate in the face to face interview, the questionnaire could be sent out by mail. In order to be able to draw appropriate conclusions from the questionnaire, it is important to consider farmers' experience, as the response from a farmer that converted to organic farming 10 years ago should be different from a farmer that recently converted to organic farming (Darnhofer *et al.*, 2005). Therefore, some of the questions developed in the questionnaire are open-ended, while others are designed as multiple choice questions with or without scaled parameters. Since the practical work of this thesis concerned Swedish wheat varieties, it would be optimal to gain knowledge from this questionnaire from Swedish organic wheat farmers. However, knowledge gained in other parts of the world from organic wheat farmers could be a benefit to Swedish farmers.

An interview technique that could be applied is, for example, ethnographic interviewing to elicit from the farmers their own ideas about all aspects of organic farming. The basic premise of ethnographic interviewing is to approach farmers in a way that acknowledges their expertise in managing a farm (Gladwin, 1989). Attention should be paid to farmers' perceptions and practices, with uncritical acceptance of their views. The aim of the interview would be to survey farmers' motivations and their reasons for actions, as well as to gain their perspectives on growing specific organic wheat genotypes with more health related compounds.

For structuring the answers from the questionnaire and interpreting them in a meaningful way, it is important to categorize the information on farmers' opinions, perceptions, and values in a systematic manner (Franzel, 1984, Murray-Prior and Wright, 1994). This can be done in both a qualitative and a quantitative manner. For analysis of farmers' responses about organic farming, in general, responses to each question should be categorized appropriately and presented quantitatively so that percentages of farmers giving similar responses can be highlighted. Based on the observations, qualitative interpretations of the responses from farmers should be done. A study of this type lends itself to providing great detail on all aspects of organic farming in a multidisciplinary fashion (Rigby *et al.*, 2001). With regards to questions related specifically to interest in organic wheat varieties with higher carotenoid contents, the same approach should be taken.

2.6 Literature review on LCA of organic wheat

A literature review of life cycle analysis of organic wheat was performed in order to assess several aspects of organic wheat farming system. For this review, socio-economic and environmental aspects were considered. Economic sustainability was evaluated in terms of energy consumption and yield. Environmental sustainability was evaluated in terms of environmental impacts, and social sustainability was evaluated in terms of farmers'/ consumers' preference on growing/ buying organic wheat. Furthermore, an attempt was made to compare outcomes of LCA of organic farming system with the outcomes of LCA of conventional farming system in order to identify potential benefits and drawbacks of each system.

3. Results

Results concerning carotenoid analysis are presented based on:

- Carotenoid amounts between four different genotypic groups, i.e. primitive wheat, old cultivars, spelt and landraces, and within each genotypic group.
- Carotenoid amount between winter and spring wheat types.
- Carotenoid amounts between 33 different organic wheat genotypes.

Carotenoid amounts in this study were analyzed by HPLC methodology, and an example of a chromatogram of genotype *Lysh vede brun borst* is shown in Figure 1.

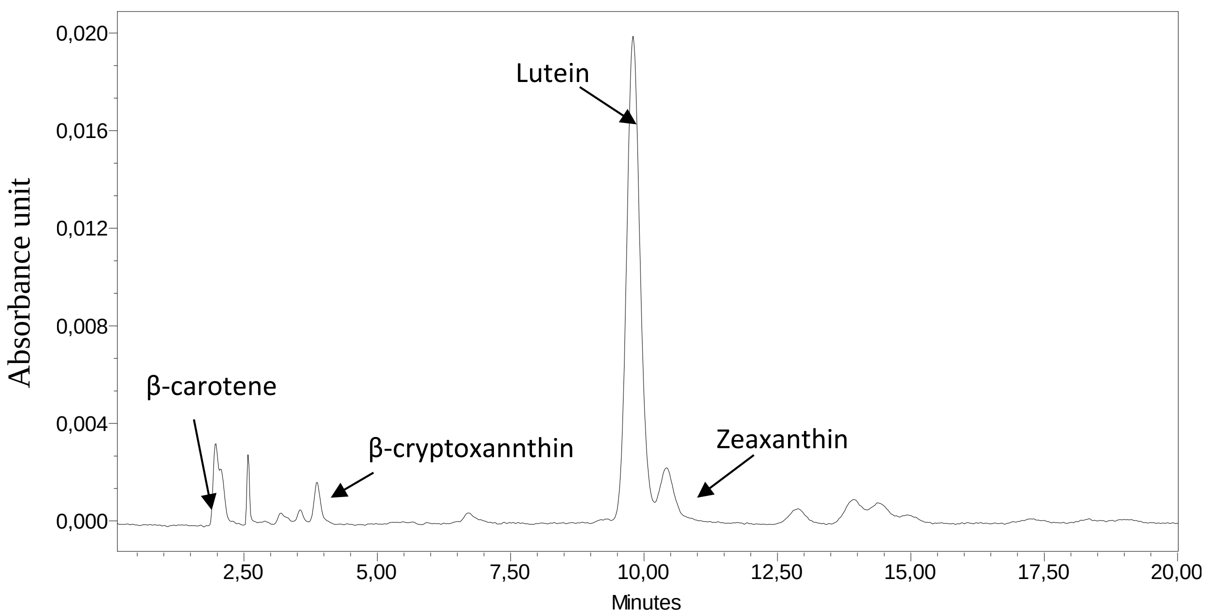

Figure 1. Chromatogram from HPLC for the studied genotype, *Lysh vede brun borst*, indicating studied carotenoid compounds. Peaks at specific time points indicate specific identities of the studied carotenoids as follows: β-carotene: 2.40 minutes, β-cryptoxanthin: 4 minutes, lutein: 10 minutes and zeaxanthin: 10.20 minutes.

3.1 Carotenoid amounts among four genotypic wheat groups

Concerning average carotenoid amounts per wheat group, no statistically significant differences were found across any group for β-cryptoxanthin, lutein, zeaxanthin, as well as total carotenoids amounts (Appendix 2). For β-carotene, *primitive wheat* was found to contain significantly lower amounts than *spelt wheat* and *landraces.* There were no statistically significant differences of β-carotene amounts between *spelt wheat*, *landraces* and *old cultivars* (Table 1).

Table 1. Variation of carotenoid amounts in four wheat genotypic groups mg/kg of DW.

Wheat groups	No. of samples	β-carotene	β-cryptoxanthin	Lutein	Zeaxanthin	Total
Spelt wheat	11	0.14(ab)	0.006(a)	1.59(a)	0.15(a)	1.89(a)
Landraces	6	0.12(ab)	0.005(a)	1.70(a)	0.17(a)	1.99(a)
Old cultivars	11	0.10(bc)	0.005(a)	1.40(a)	0.17(a)	1.67(a)
Primitive wheat	5	0.02(c)	0.001(a)	1.39(a)	0.18(a)	1.60(a)

Note: Means that do not share the same letter are significantly different as determined by one way ANOVA with Tukey HSD analysis.

The range of total carotenoid amount for each group is as follows: within *landraces* 1.02-4.08 mg/kg (*oland 5- oland 8*) (Appendix 3), *spelt* wheats 1.38- 2.50 mg/kg (*6356 Spelt- Schweiz*) (Appendix 4), *old cultivars* 0.78-2.48 mg/kg (*Fylgia 1- Aura*) (Appendix 5) and within *primitive* wheats 1.11-2.03 mg/kg (*T.polonicum- Rauweizen*) (Appendix 6).

3.2 Carotenoid amounts between spring and winter wheat

Carotenoid amounts observed in spring and winter wheats are presented in Table 2.

Table 2. Variation of carotenoid amounts based on spring and winter wheat types mg/kg of DW.

Wheat type	No. of samples	β - Carotene	β-Cryptoxanthin	Lutein	Zeaxanthin	Total
Spring wheat	10	0.104(a)	0.005(a)	1.32(a)	0.19(a)	1.62(a)
Winter wheat	23	0.103(a)	0.004(a)	1.60(a)	0.16(b)	1.86(a)

Note: Means that does not share the same letter, are significantly different as determined by student's t test analysis.

In this study, *spring* wheat showed significantly higher amounts of zeaxanthin in comparison with *winter* wheat (Table 2). Otherwise, no statistically significant differences were found between *spring* and *winter* wheats for β-caroten, β-cryptoxanhin and lutein (Table 2).
In comparing average amounts of the individual carotenoids measured in this study, lutein was the highest in both *spring* and *winter* wheat genotypes, while, β-cryptoxanthin was the lowest (Table 2).
Between the studied genotypes of *spring* wheat, the significantly highest total amount of carotenoids was observed in *Oland 8* (4.08 mg/kg of DW) and the significantly lowest amount was found in *Flygia 1* (0.78 mg/kg of DW) (Figure 2a and Appendix 7). For *winter* wheat the significantly highest total amount of carotenoids was observed in both *Aura* and *Schweiz* (2.5 mg/kg of DW, each) and the significantly lowest amount was found in *T.polonicum* (1.10 mg/kg of DW (Figure 2b and Appendix 7).

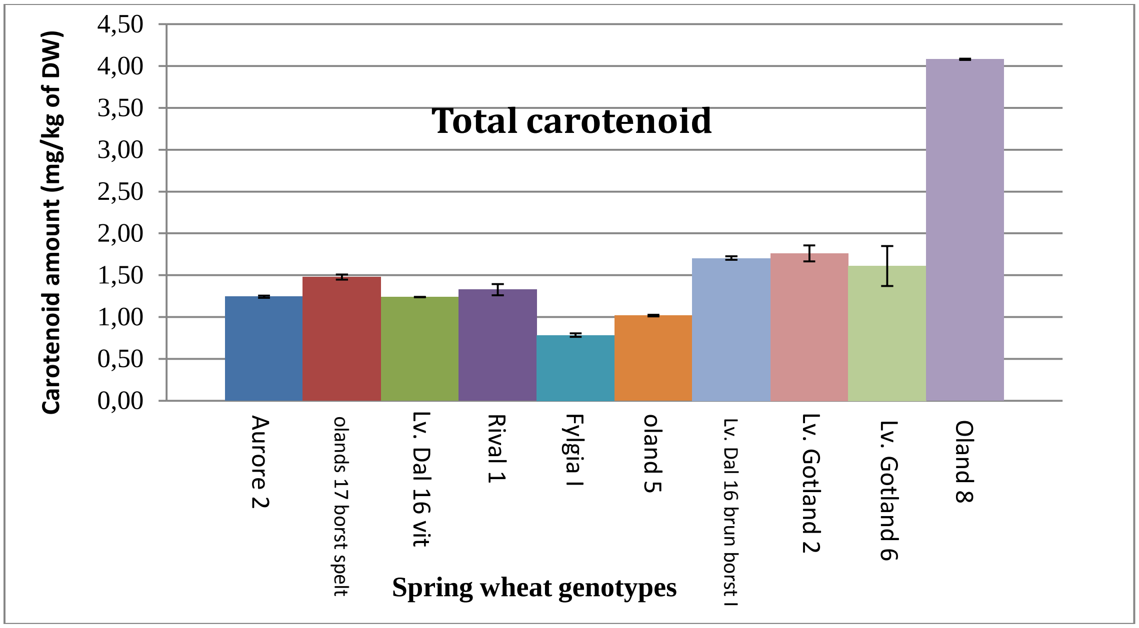

Figure 2a. Total amount of carotenoids among spring wheat genotypes (*n*=10). Error bars show standard deviation.

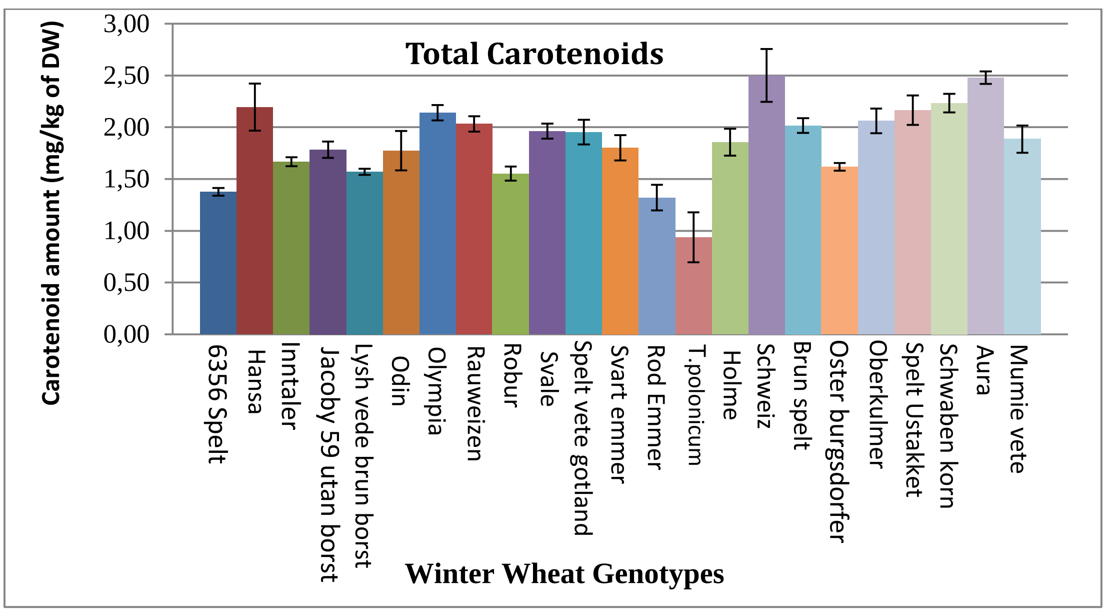

Figure 2b. Total amount of carotenoids among winter wheat genotypes (n=23). Error bars show standard deviation.

3.3 Range of carotenoids among organically grown wheat genotypes

Between the studied genotypes the amounts of β-carotene, β-cryptoxanthin, lutein and zeaxanthin ranged from (0.20-0.28) mg/kg of DW, (0-0.02) mg/kg of DW, (0.06-3.70) mg/kg of DW and (0.08-0.27) mg/kg of DW, respectively (Appendix 7). Furthermore, it was observed that the mean amounts of each individual carotenoid across all genotypes were significantly different from the mean amounts of every other individual carotenoid and total carotenoid amounts (Appendix 8).

Figure 3 shows the distribution of the studied carotenoids among 33 organically grown wheat genotypes. The highest amounts of β-carotene were observed for the genotypes *Aura* (0.28 mg/kg), *Jacoby 59 utan borst* (0.26 mg/kg), *Oland 8*(0.23 mg/kg) (Figure 3a).

The highest amounts of β-cryptoxanthin were observed for *Aura* (0.02 mg/kg), *Oland 8 (*0.01 mg/kg) and *Jacoby 59 utan borst* (0.01 mg/kg) (Figure 3b).

For lutein, the highest amounts were observed for genotypes *Oland 8* (3.70 mg/kg*), Schweiz* (2.21 mg/kg) and *Aura* (2.04 mg/kg) (Figure 3c).

For zeaxanthin, the highest amount were observed for genotypes *Lv. Gotland 2* (0.27 mg/kg), *Oland 5* (0.27 mg/kg), and *Rival 1* (0.26 mg/kg) (Figure 3d).

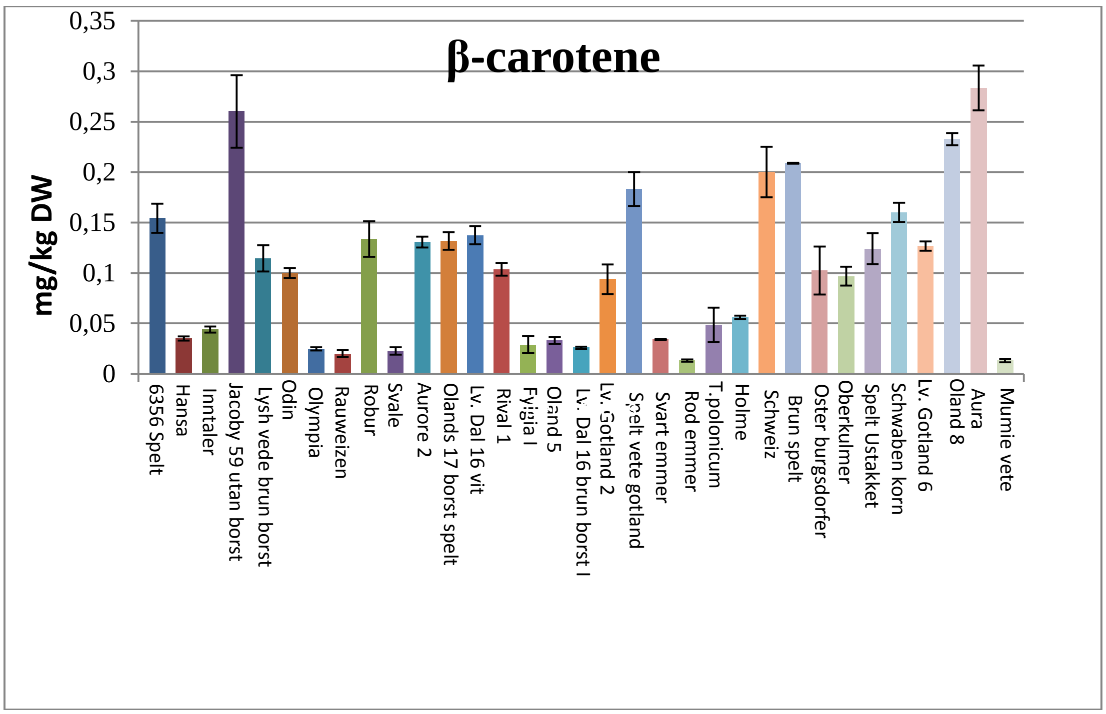

Figure 3a. Distribution of β-carotene amounts among 33 organic wheat genotypes. Error bars show standard deviation.

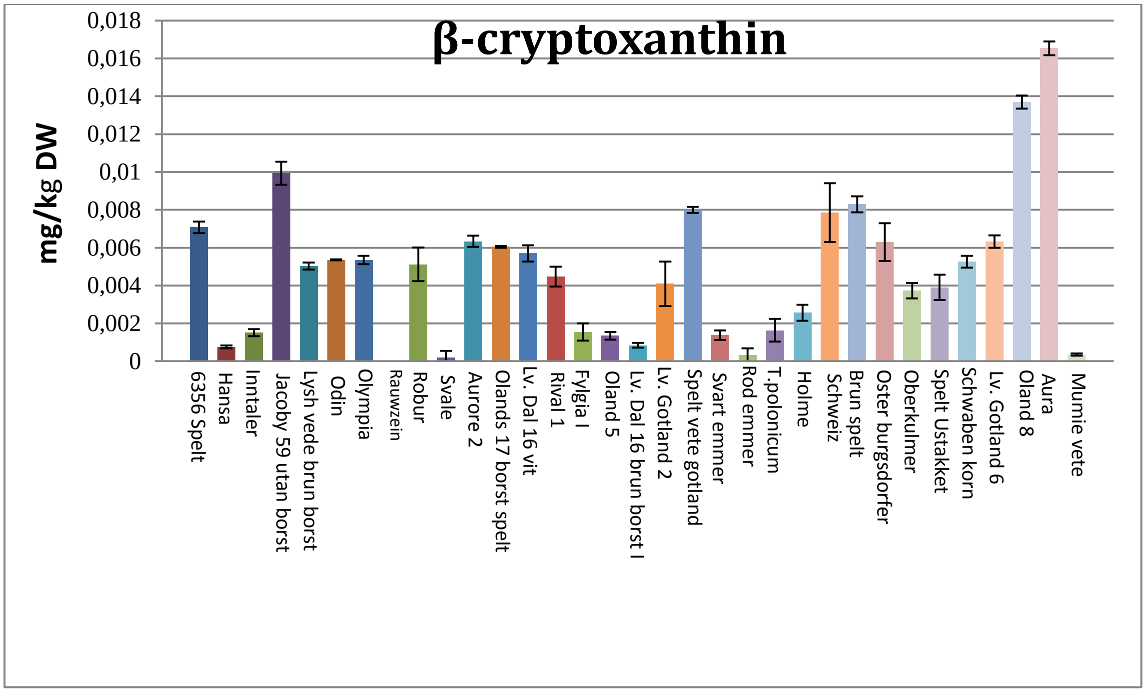

Figure 3b. Distribution of β-cryptoxanthin amounts among 33 organic wheat genotypes. Error bars show standard deviation.

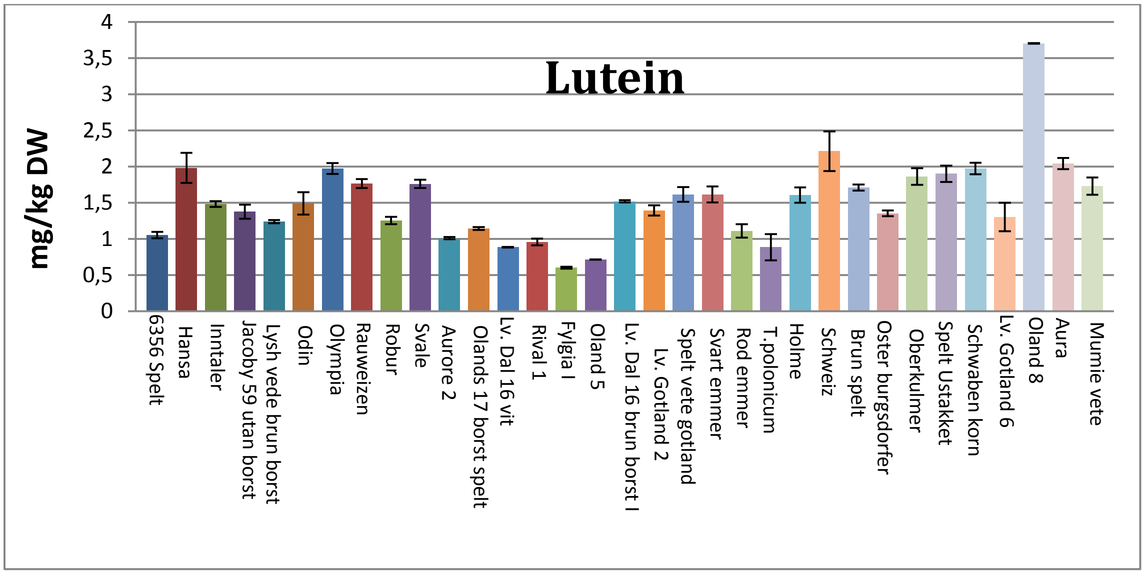

Figure 3c. Distribution of lutein amounts among 33 organic wheat genotypes. Error bars show standard deviation.

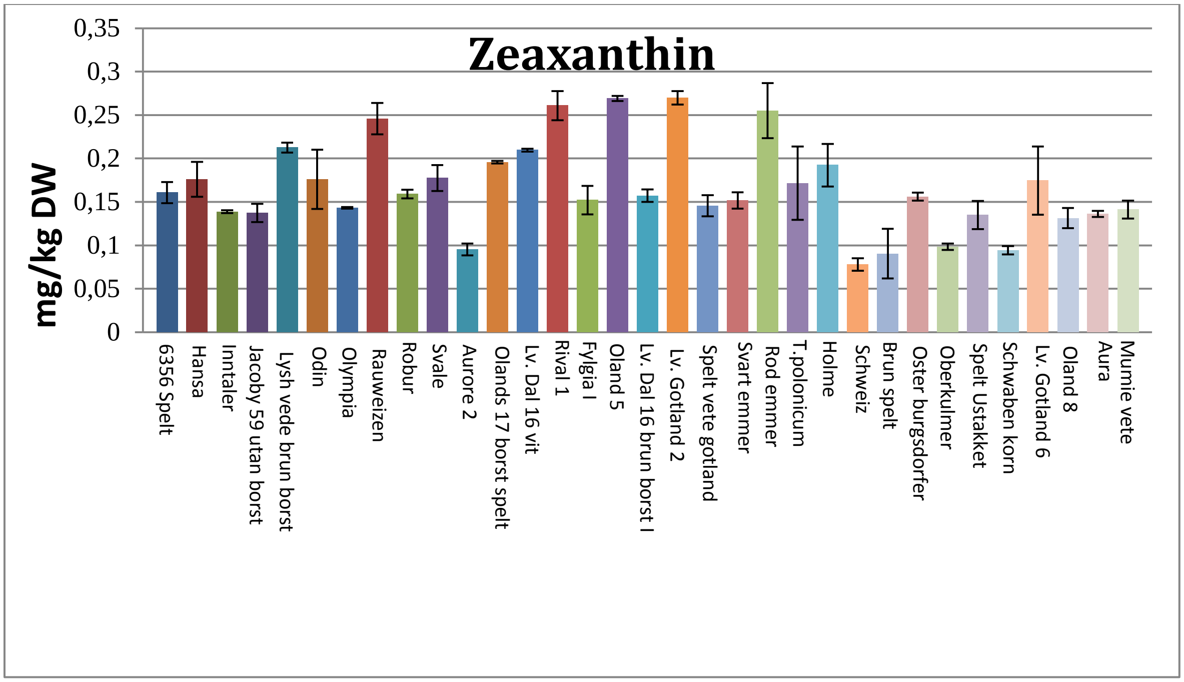

Figure 3d. Distribution of zeaxanthin amounts among 33 organic wheat genotypes. Error bars show standard deviation.

Total carotenoids, β-carotene, β-cryptoxanthin, lutein and zeaxanthin all showed variation in their amounts as evaluated within all 33 samples (Figure 3, Appendix 7). For total amounts of carotenoids, genotypes *Oland 8* (4.08 mg/kg), *Schweiz* (2.49 mg/kg) and *Aura* (2.47 mg/kg) were the significantly highest among all 33 genotypes.

3.4 Comparison of carotenoid amounts with other studies

Results from this study were compared with similar studies on carotenoid content for conventionally grown wheat samples from the literature (Table 3).

Table 3. Average levels of carotenoids in 33 different genotypes of organic wheat compared with other studies (mg /kg of DW).

No. of samples	β-carotene	β - cryptoxanthin	Lutein	Zeaxanthin	Total	References
33	0.10±0.06	0.005±0.0044	1.51±0.58	0.16±0.048	1.78±0.6	Present study
8	0.16±0.00	n.d	1.003±0.02	0.28±0.00	1.44±0.005	(Moore *et al.*, 2005)
4	0.06±0.00	0.23±0.02	1.19±0.02	0.33±0.002	1.81±0.007	(Zhou *et al.*, 2005)
7	0.19±0.03	0.44±0.01	0.98±0.02	1.24±0.00	2.85±0.01	(Zhou *et al.*, 2004)
14	n.d	n.d	2.65 ±0.65	0.26 ±0.04	2.91±0.72	(Panfili *et al.*, 2004)

n.d. Indicates that the data was "not determined" in that particular study.

According to Table 3, the amounts of total carotenoids in this study are not higher as compared with other studies. The highest total carotenoid amount is seen in the study by Panfili *et al.*, (2004).

3.5 Life cycle analysis (LCA) of growing organic wheat

The second aim of this study was to analyze several aspects of profitability and sustainability of organic wheat farming systems through a literature review of LCA with respects to economy, environment and social sustainability. To achieve this, a summary of scientific literature on this topic has been included.

3.5.1 Economy

Economic sustainability of organic production has been discussed in this thesis through literature review based upon two main elements, energy consumption and profitability (net returns, yield and market). These parameters have been chosen because without profitability, ultimately organic farming systems will not achieve large-scale, long-term viability. Additionally, energy consumption in terms of fuel for all farming practices contributes significantly to total cost inputs of any farming system (Pimentel *et al.* 2005).

- Energy

In a study by Pimentel *et al.* (2005) it was reported that, about 5.2 million kcal of energy per ha were invested in the production of cereals in the conventional system. The energy inputs for the organic systems were found to be significantly less than those of the conventional system (Smolik *et al.*, 1995). In another study on winter wheat production, in terms of energy consumption per unit of land and per unit of product, it was observed that the organic system used significantly lower amounts of energy than the conventional system (Tuomisto *et al.*, 2012).

- Profitability

Different results have been observed for profitability of the two systems of organic and conventional farming. In a study by Pimentel *et al.* (2005), it was observed that the economic returns for organic cereal production were slightly higher (2%) than economic returns of conventional cereal production. In the Netherlands, organic cereal production systems were reported to have a net return of 953 Euros/ha compared with conventional cereal production systems, 902 Euros/ha (Pacini *et al.*, 2003).

Furthermore, in another study it was observed that although inputs for the organic and conventional farming systems were different, the overall economic net returns were similar (Pimentel *et al*., 2005). On the other hand, Dobbs and Smolik, (1996) reported a 38% higher gross income for the conventional system as compared to the organic system. In a study of organic wheat in the U.S, it was shown that total economic costs per bushel were higher in organic system than conventional and even with higher organic price premiums, profit margins remained higher in the conventional system (McBride *et al*., 2012).

In terms of yield, it is generally observed that conventional farming brings higher yields. Yields of winter wheat from organic and conventional farming have been compared in Meisterling *et al.*, (2009) indicating that yield is higher in conventional system (9168 kg of DM) than organic system (4747 kg of DM; Table 5). In another study, European field tests indicate that organic wheat and other cereal grain yields are on average 30% to 50% lower than conventional cereal grain production (Maeder *et al*., 2002). From a comparison of manure-based organic growing system and synthetic chemical fertilizer-farming system, wheat yields were not significantly different (3.40 t/ha versus 3.45 t/ha) (Pimentel *et al*., 2005). It should be noted that per unit of product, in organic wheat farming, land use is significantly higher than in the conventional system, even when not considering extra land allocation for production of green manure (Tuomisto *et al.*, 2012).

The total organic agricultural land area worldwide (37.2 million hectares in 2011) has remained stable between 2010 and 2011. In Europe, however, organic farmland increased by 6.3 percent from 2010 to 2011. From 1999 to 2011 the total organic agricultural land increased from 11 million ha to 37.2 million ha worldwide, while in Europe it increased from 3.66 million ha to 10.64 million ha during the same time period. Organic agricultural lands worldwide are as follows: Africa 3%, North America more than 7%, Asia 10%, Latin America 18%, Europe 29% and Oceania 33% of the world's organic agricultural lands. Sweden was among the countries with the highest share of total organic agricultural land in 2011 at 15.4% (FiBl and IFOAM, 2013).

For arable crops including cereals, the growth of organic farmlands was from 3.4 million ha in 2004 to 6.3 million ha in 2011 worldwide. Of the 6.3 million ha of total arable organic farmland, 40% is for growing cereal crops including wheat. The development of organic land uses for cereals has increased from 1.5 million ha to 2.5 million ha between the years of 2004-2011. From 2010 to 2011, there was a 4% increase in organic agricultural lands for cereal crops (FiBl and IFOAM, 2013).

In 2011, the leading countries in organic market size were U.S (29 billion USD), Germany (9.2 billion USD) and France (5.2 billion USD) which contributes to 63 billion US dollars of the total market in the world. This is a dramatic increase from 15.2 billion US dollars in 1999 (FiBl and IFOAM, 2013).

3.5.2 Environmental impacts

There are environmental costs associated with use of synthetic chemical fertilizers and pesticides (Pimentel *et al.*, 2005). In different studies, soil organic matter has been found to be significantly higher in the organic system than in the conventional system (Pimentel *et al.*, 2005, Marinari *et al*., 2006). Conversely, some other problems identified with the organic system include nitrogen deficiency, pest control and weed competition (Clark *et al*., 1999, Pimentel *et al*., 2005).

Regarding environmental costs of organic farming, it was found in one study that local road transport from farm to point-of-sale includes the main part of externalities (29%) (Meisterling *et al.*, 2009). The global warming potential (GWP) impact of producing 0.67 kg of conventional wheat flour (for a 1 kg bread loaf), not including product transport, is 190 g CO_2-eq, while the GWP of producing the wheat organically is 160 g CO_2-eq (Figure 4). But, when organic wheat is shipped further than 420 km from farm gate to the point-of-sale, organic and conventional wheat systems have similar GWP impacts (Figure 4; Meisterling *et al.*, 2009). Machinery manufacturing impacts are the same for the two systems of farming, however organic wheat cultivation demands increase tillage. Another study on environmental impact in winter wheat production showed that the organic system had significantly lower GWP per hectare as compared to the conventional system, but in terms of unit of product, the GWP was not significantly different between the two systems (Tuomisto *et al.*, 2012).

Through measurements of nitrogen (N) leaching and volatilization from organic and conventional wheat farms, it was observed that nitrous oxide (N_2O) emissions are similar for both farming systems (1.4% versus 1.3% respectively). However, if N_2O emissions as a percentage of supplied N reach 2% for the organic farming system, but in conventional systems is maintained at 1.3%, the conventional wheat system would have a lower GWP impact than organic wheat (Meisterling *et al.*, 2009). The difference of N_2O emissions between organic and conventional system could be due to inefficient utilization of organic fertilizers by the crop systems (Dahlin *et al.*, 2005, Meisterling *et al.*, 2009). The GWP and transport differences are shown in Figure 4.

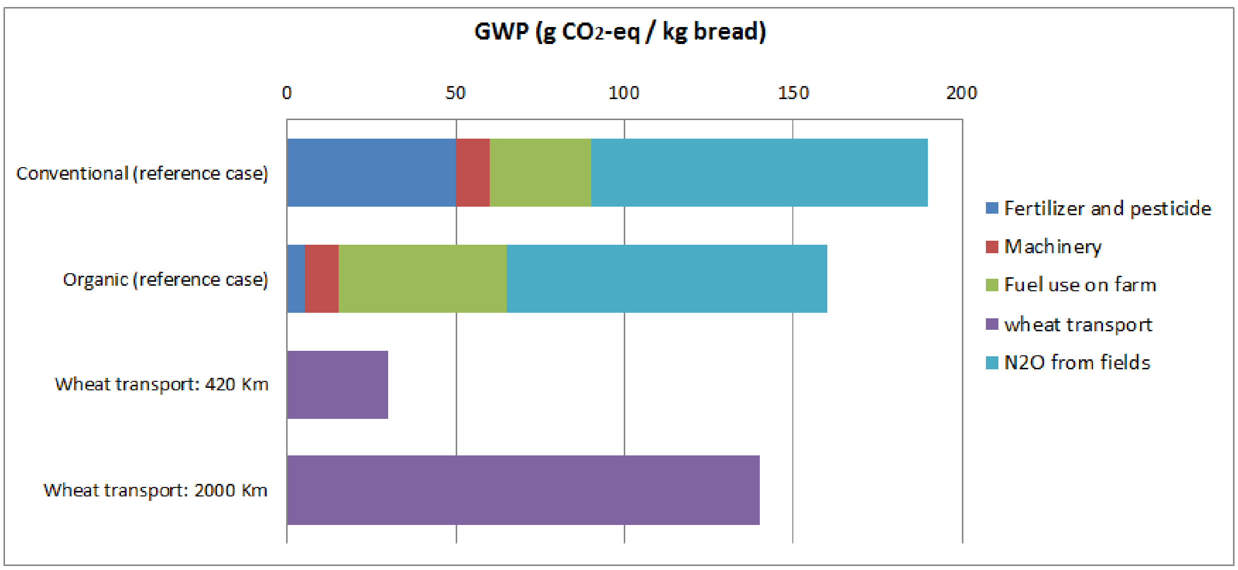

Figure 4. *Global warming potential of wheat production*. The top two bars show GWP of wheat production without shipping and the two bars at the bottom show GWP associated with two transport distances; 420 km and 2000 km. Transport considered 50/50 truck/train. Figure adapted from Meisterling *et al*., 2009.

3.5.3 Farmers' interview

To be able to understand farmers' motivations to grow/consume organic wheat, a literature review was conducted based on published reports involving questionnaire based interviews with farmers. The results of this review were reported keeping in mind the questions from the questionnaire designed in this thesis. The reason for the literature review was to improve the understanding of farmers' beliefs and motivations that influence their choice of farming system, as well as their concerns for production of healthy food products from cereals e.g. wheat.

There are many reasons for farmers to choose organic production (Fairweather, 1999). Important motivations for organic farmers include the following: concern for the environment, chemicals in food, health awareness as consumers who eat their products, better taste, farmers' experience of illness from chemicals, production of higher value crops, satisfaction with organic fertilizers, high price of chemicals, and better soil quality (Yunlong and Smit, 1994).

A study by Fisher, (1989), in New Zealand, shows that many farmers who adapted organic farming did so because they see organic farming as a profitable, low-input system and as an environmentally-healthy alternative. Birzer and Badgery (2006), found in a survey that organic farmers who had previously performed conventional farming noted a decline in yield after switching to organic farming; whereas farmers who already used mainly organic methods did not see a drop in yields in several years. However, farmers' motivations for choosing organic farming in developed countries such as Denmark, Sweden, Germany, Ireland, U.S.A, Canada, Korea, Australia and New Zealand were not only related to economical benefits. For these organic farmers, concerns about environment (effects of synthetic chemicals, decline in soil fertility, water and soil pollution), food quality, health of themselves and their families and consumers, no use of chemical fertilizers were some of their main motivations for choosing the organic farming method (Rigby *et al.*, 2001).

As shown in Figure 5, the leading reasons for farmers to choose organic farming are related to environment and health. Indeed, a significant percentage of farmers cite their own health or the health of consumers as the main reason for a switch to organic production; it was reported that this is often a result of a negative personal experience with ill health due to chemical exposure (Fairweather, 1999).

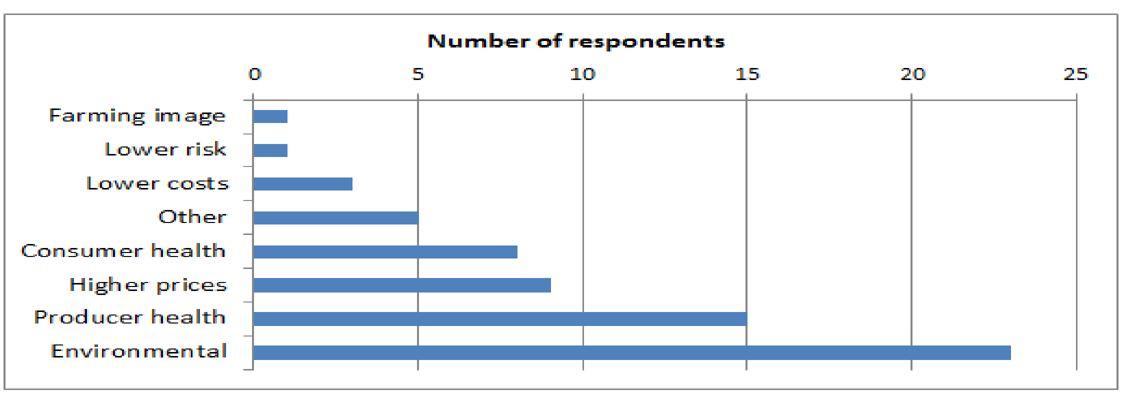

Figure 5. Most important factor in farmers' decision making to produce organically. Adapted from Rigby *et al.*, 2001.

In an example from a developing country, findings suggest that wheat farmers are not as interested as other farmers in practicing sustainable agriculture. In Iran, there is a great concern with wheat yield stability and increase in yields because wheat is the major food source. Agricultural extension organizations in Iran have not been a primary information source on organic farming for farmers. So, wheat farmers perceived that several courses on the use of chemical fertilizers, pesticides and insecticides, use of agricultural machineries and tillage, would be the most useful agriculture extension instruction (Chizari *et al.*, 2001).

However, in order to reach an understanding of why farmers grow or do not grow organic, one must consider other factors in addition to their motivations, such as reasons to actively reject organic farming, known as rejection principles (Appendix 9). Some rejection principles include: farmers' low level of knowledge and education on organic agriculture, lower yields and financial benefits, government regulations, requirement of more land to grow the world's food, reliance on fossil fuel, receiving weedy crops instead of the main crop (Rigby *et al.*, 2001). There are also financial concerns behind farmers' decisions to reject or accept organic farming. National or regional agricultural programs for organizing subsidy schemes are mostly supportive towards large-scale, chemically intensive agricultural systems that artificially lower the price of conventional products (IFOAM, 2008).

4. Discussion

For this study, it was hypothesized that higher amounts of carotenoids will be present in older genotypes of wheat in comparison with modern wheat varieties. Based upon the results of this study, the hypothesis was validated for several specific wheat genotypes studied. Although general carotenoid concentrations in wheat endosperm are known to be relatively low, there exists significant variation across genotypes (Howitt and Pogson, 2006). In our study, the genotypes Oland 8 (4.08 mg/kg), Schweiz (2.49 mg/kg) and Aura (2.47 mg/kg) showed potential as promising genotypes, in terms of promoting human health due to the currently low daily intake of lutein (1.5-2 mg) compared to the recommended doses (5-6 mg/day) (Abdel-Aal *et al*., 2007). Variation in carotenoid content across genotypes could be due to several factors. In addition to this study, significant genetic variation in carotenoid amounts has been documented, with variable ranges reported for durum varieties as compared to bread wheat varieties (Hentschel *et al*., 2002; Zandomeneghi *et al*., 2000). Howitt and Pogson (2006) reviewed carotenoid biosynthesis pathways, including genetic components and regulatory mechanisms. Accordingly, one source of genetic variation in carotenoid amounts could be attributed to differential regulation of the carotenoid biosynthetic pathways during grain filling. Also, wheat grain contains several enzymes that are known to be involved in the destruction of carotenoid pigments, for example during dough mixing. Enzymes, such as lipoxygenase, polyphenol oxidase and peroxidase are highly concentrated in bran layers (Rani *et al.*, 2001), and in this study we have used whole meal flour containing bran. Secondly, the storage conditions, before the extraction procedure was performed, may have led to some carotenoid degradation (Davey *et al*., 2009, Hidalgo and Brandolini, 2008).

This study represents a first examination of carotenoid content in these specific organically grown wheat genotypes and provides a basis for future studies. It will be essential to identify the source of this variation, whether it be environmental or genetic, as well as genetic-environment interactions, in order to maximize carotenoid amounts and thus the potential benefits derived from carotenoids for human health.

4.1 Carotenoid content in individual genotypes

Four carotenoid compounds (β-carotene, β-cryptoxanthin, lutein and zeaxanthin) were examined based on previous studies that demonstrate the prevalence of these compounds in wheat (Lepage and Sims, 1968). The findings of this study show that there are significant differences in carotenoid contents between specific genotypes (Appendix 7), indicating that there is potential for genetic variation concerning, for instance, specific carotenoid amounts e.g. lutein. In order to completely determine the source of this and other carotenoids variation one must consider genetic effects within each genotype as well as environmental effects, or interaction of both. Difference in genetic variation could also be related to regulation of factors both upstream and downstream of lycopene, an important intermediate in the biosynthesis of many carotenoids (Howitt and Pogson, 2006).

In terms of average total carotenoid amounts per sample, the results of this study are consistent with the results of other studies that examined carotenoid contents in conventional wheat (Moore *et al*., 2005, Zhou *et al*., 2005, Zhou *et al*., 2004). The genotypes identified as having the highest amounts of carotenoids (Appendix 7) could thus be of interest for use in future plant breeding programs to produce organic wheat, with higher nutritional values.

4.2 Carotenoid content between wheat groups

Regarding variation of carotenoid amounts among the wheat groups, the greatest variation across genotypes was seen in the *landraces* group (Appendix 3), indicating that genetic differences for carotenoid synthesis could exist within this group. Genetic sources of variation of carotenoid content should be examined to determine the role of genetic versus environmental effects, as well as interaction of both on carotenoid amounts. Our examination of individual carotenoid amounts as a factor of wheat group showed relatively few differences across groups. The reason for this is most likely attributable to variation across genotypes within all groups, which leads to similar mean carotenoid amounts across all groups.

A common biosynthetic pathway for carotenoids exists in nearly all plant species, including wheat (Howitt and Pogson, 2006). Genes that encode enzymes involved in carotenoid synthesis should be considered primary targets of investigation concerning genetic effect on carotenoid amounts in different wheat genotypes. Genes involved in the degradation of carotenoids should also be considered (Leenhardt *et al.*, 2006). It would be of interest to determine if significant genetic effects on carotenoid amounts can be found in these genotypes, in terms of the carotenoid biosynthesis and degradation genes, and identify the variants of those genes that correlate to the highest carotenoid concentrations (Howitt *et al.*, 2009).

It should be noted here that for all groups, lutein consisted of between 84% and 87.7% of total carotenoid amount, as was also found in other studies (Abdel-Aal *et al*., 2007, Konopka *et al*., 2006, Lepage and Sims, 1968) (Appendix 7). Thus, lutein composition factored significantly in total carotenoid amount. Accordingly, as there were no statistically significant differences across any wheat groups concerning mean lutein amounts, there were also no statistically significant differences across any wheat groups for the summed mean of all carotenoids examined.

On the other hand, in other studies that compared carotenoid amounts in *primitive* wheats versus common bread wheat, *primitive* wheats consistently had significantly higher amounts of carotenoids (Abdel-Aal *et al*., 2007, Leenhardt *et al*., 2006). In case of these differences in carotenoid amount between *primitive* and common bread wheat, a genetic component is expected to contribute to variation in carotenoid content across groups. In order to exploit genetic diversity and maximize individual carotenoid component amounts within one genotype of wheat, innovative breeding programs utilizing genotypes with the highest amounts of individual carotenoids from different wheat groups could be undertaken.

These results could be contrasted to a study done by Hussain *et al.* (2010), which examined the mineral content of wheat genotypes similar to those used in this thesis. In that study, varieties of *primitive* and *old cultivars* contained the highest amounts of minerals among several groups examined. In this study, however, no statistically significant differences were found in terms of total carotenoid amounts between *old cultivars, primitive, landraces* and *spelt* wheat groups. It should be mentioned that this study examined 5 genotypes of *primitive* wheat and 11 genotypes of *old cultivars,* whereas Hussain *et al.* (2010) examined 42 genotypes of *primitive* wheat and 206 genotypes of *old cultivars*. Low sample size in this study compared to high sample size in the one by Hussain *et al.* (2010) could affect the qualitative nature of the results, because only a small part of the genetic diversity may be sampled (Lampi *et al.*, 2008).

In order to draw accurate conclusions from the comparisons made across these two studies, a larger sample size should be examined for carotenoid content. Nonetheless, it can be said that conclusions about the total nutritional quality of specific genotypes and groups cannot be made based upon the assessment of one specific kind of nutritional component. The whole picture must be taken into consideration (Šramková *et al.*, 2009).

4.3 Carotenoid content between wheat types

In comparing *winter* and *spring* wheats, *spring* wheat contained higher amounts of zeaxanthin (Table 2). Total carotenoid amounts were not significantly different between *winter* and *spring* wheat genotypes. However, in a study by Konopka *et al.* (2006) it was observed that spring wheats contained higher carotenoid amounts than winter wheat.

In a study examining Swedish adapted organic wheat genotypes, it was observed that *spring* wheats generally contained higher mineral contents than *winter* wheats (Hussain *et al.*, 2010). This was attributed to higher yields in *winter* wheats causing a dilution effect. However, in this study, there was no significant difference between total carotenoid amounts observed in *winter* and *spring* wheats. In this case, a dilution effect is not expected. While minerals are absorbed from the soil, carotenoids are synthesized by the plant, so an accumulation effect is expected (Richards, 2000). Higher yields (as occur in winter wheats) should normally correlate to increased amounts of the synthetic enzymes involved in carotenoid synthesis and thus accumulation of greater amounts of carotenoids. However, other factors may be involved that could affect differences in carotenoid amounts between spring and winter wheats. It must be noted that there was a trend towards higher total carotenoids in winter wheats, but there was not statistical support for this difference. Importantly, in this study differences in sample size between *spring* wheat (n=10) and *winter* wheat (n=23) may reflect a lack of statistical significance between the two wheat types. Further experimentation should be conducted to identify the source of these differences in order to be able to more fully evaluate the relative nutritional quality of spring versus winter wheat.

Treatment effects on carotenoid content

It is important to mention that for carotenoid analysis, several important points should be considered. Sample storage conditions can significantly affect nutritional quality. Immediate sample storage at low temperature (-70℃) after harvest have been recommended for ideal storage conditions (Hidalgo and Brandolini, 2008, Leenhardt *et al*., 2006). A recent study shows that carotenoid extracts even in the darkness and low temperature (-20˚C) had breakdowns of around 5% per day (Davey *et al.*, 2009). Moreover, studies on the evaluation of loss during saponification showed considerable amount of loss of carotenoids (Oliver *et al.*, 1998). In our study, wheat samples were stored in a room temperature for certain amount of time while threshing was completed. This fact unavoidably suggests that storage time could negatively affect the amount of carotenoids, therefore, the amounts that we observed were possibly lower than they would otherwise be without significant storage time at room temperature.

4.4 Carotenoid content: organic versus conventional wheat

Carotenoid amounts from this study were compared with amounts of modern conventional bread wheat from other studies (Table 3).

Total carotenoid amounts from this study were not the highest among four other studies on carotenoid amount of conventional wheat (Table 3). These results could indicate that variation between carotenoid amounts is not only affected by farming systems, other aspects, including genetic and environmental factors, should be taken into consideration.

In a further comparison among the present study and a study of conventional wheat (Lachman *et al.*, 2012), it was determined that in *spring* wheats, lutein (1.31 mg/kg DW versus 1.10 mg/kg DW) and zeaxanthin (0.19 mg/kg DW versus 0.14 mg/kg DW) amounts were greater in this study while β-carotene was higher in conventional wheat (0.11 mg/kg DW versus 0.12 mg/kg DW). Whereas in comparing *Emmer* wheats, lutein (1.36 mg/kg DW versus 0.76 mg/kg DW) and zeaxanthin (0.20 mg/kg DW *versus* 0.14 mg/kg DW) amounts were higher in this study as compared to the conventional varieties from Lachman *et al.*, (2012).

It should be noted here that these comparisons between organically and conventionally grown wheat genotypes are indirect based upon literature review. Farming system type is not the only factor to be considered for completely valid considerations of nutritional quality. Genotype and growing environment must also be controlled to be able to draw appropriate conclusions. These comparisons were made only to suggest that differences between the two farming systems could exist. However, in a study by Langenkamper *et al.* (2006) it was suggested that, based upon examination of several nutritional factors (such as antioxidative capacity, phenol content, fiber levels, etc.), wheat from one or the other system of agriculture would not be better or worse regarding nutritional value.

It was mentioned by Hussain *et al.* (2012b) that genotype, growing environment, and interaction between genotype and environment may significantly affect the chemical compositions and antioxidant activities of soft wheat flour. In this study, genotypic effect in Swedish adapted varieties of wheat was examined. However, growing environment factors, including local climate and soil qualities were not considered. In the study by Hussain *et al.* (2012b) it was concluded that genotype had less influence on both chemical composition and antioxidant activities compared to environment and the interaction between genotype and environment. Therefore, these growing environment factors must be given future consideration to more fully determine optimal wheat varieties to be selected for expanded breeding programs in consideration of carotenoid contents. Only then, should it be possible for wheat breeders to select the optimal environment and genotype to improve the levels of selected health components and antioxidant activities of wheat (Lv *et al.*, 2013).

4.5 Life cycle analysis

The results summarized from literature review on LCA of organic wheat farming were compared with the results summarized from literature review on LCA conventional wheat farming to analyze benefits and drawbacks of the two systems. Agricultural sustainability was the objective with respect to economy, environment and social perspectives. The aim of this comparison targets the productivity of the organic system while balancing the economic costs against environmental, social and health considerations.

4.5.1 Economical sustainability

Evidence suggests that in terms of economical sustainability there is little difference between the two systems of farming. It has been mentioned by Nemes (2009) that if organic farming offered an enhanced environmental quality, and healthier cereal products, but not offering adequate economic profits to the farmers, it would obviously remain a luxury method of cereal production practiced by a small portion of farmers (Nemes, 2009).

As conclusions drawn from this literature review indicate, economical benefits between two systems of farming are not always consistent. In some case studies organic farming brought higher economic returns, whereas in others, conventional farming was more profitable or similar. Organic farming could bring economical benefits, if it relies on locally available production inputs. Additionally, local organic farming is less dependent on fossil fuel availability and also has less input prices. Input prices will be different in two systems, with regards to fertilizers and pesticide, plough and tillage, seeds, manure, herbicides, energy, fuel, labor, machine repair and maintenance, transport and irrigation expenses (Nemes, 2009). Reasons for these differences can be varied. For instance, weed control in organic agriculture may include manual removal or mechanical tillage, plastic mulching and flaming; these costs will not be considered if only herbicide costs are accounted for (Nemes, 2009).

- Energy

Both organic and conventional wheat production systems consume an equal amount of liquid fuel and use farm equipment at similar proportions per hectare. However, there are differences between the two systems per unit of production (Meisterling *et al.*, 2009).

The intensive requirement of fossil fuel energy of the conventional farming system for nitrogen production increases the overall energy inputs in conventional compared to organic production systems. Fossil fuel is also required for transport and application of the manure to the field in conventional system (Pimentel *et al.*, 2005). Furthermore, in conventional system, the production and transport of pesticide and herbicide can also have a significant effect on total energy inputs.

However, organic wheat production is not always more sustainable than conventional wheat production in terms of energy usage, as it is not uncommon for the final product to travel farther from farm gate to point-of sale in the organic system as compared with conventional system (Meisterling *et al.*, 2009). The highest energy use reductions in organic systems are realised through the replacement of synthetic nitrogen fertilisers with organic alternatives i.e. legumes and/or cattle manure, and also by using no-tillage instead of ploughing (Tuomisto *et al.*, 2012). In organic farming, mechanical weed control is used instead of using herbicides, so there is no energy use regarding production and transport of herbicide, but there will be additional costs regarding hiring labour.

- Profitability

Increased profitability of organic agriculture depends on several aspects, e.g. price, net returns, yield, consumer demand and market (Nemes, 2009). Higher selling prices in the organic market could make organic products more profitable, even with lower yields (Dobbs *et al*., 1998, Chavas *et al*., 2009). Lower yields for the organic compared with the conventional systems appear to be a result of lower nitrogen-nutrient inputs in the organic systems, as there may be nitrogen shortages that may reduce crop yields temporarily. But over time, these can be overcome by elevating the soil nitrogen level through the use of organic cropping systems (animal manure and/or legume cropping) (Pimentel *et al*., 2005). Alternatively, yield similarities or differences between the two systems may be influenced by differences in regions and climate, so there might be differences in yields of specific genotypes of this study if grown in different part of the world (Smolik *et al*., 1995).

Net returns of organic wheat production could increase by higher price premiums and decreased input and operating costs of farming. Also, market of organic cereal products is increased as people become more aware of healthy foods and their role in their life (Hughner *et al.*, 2007). Organic wheat production will not be successful unless wheat farmers can become competitive in the markets to which they sell. In order to increase organic product markets, it is necessary to understand what motivates consumers to buy organic products and redirect public support towards sustainable practices (Hughner *et al.*, 2007). Therefore, if there is a desire to promote organic products, governments could be an important element to implement consumer awareness programs towards buying organic food in the current food market.

4.5.2 Environmental sustainability

Organic farming could be more sustainable than conventional farming, if we consider only environmental aspects such as soil fertility, organic matter content and biological activity, lower water pollution as well as the production of wheat free from synthetic chemicals. It was concluded from some studies that although organic wheat production has potential environmental benefits (Pimentel *et al.*, 2005), there is information about environmental impacts associated with production and transport of the wheat in organic system (Meisterling *et al.*, 2009; Figure 5). For example it was indicated that if organic wheat is transported 420 km further than

farm gate to point of sale, GWP would be similar in both systems (Meisterling *et al.*, 2009). Furthermore, it was shown that the utilization of less fossil fuels and energy conservation by the organic farming contributes to lower amounts of carbon dioxide (CO_2) released into the atmosphere, and thus the problem of global climate change (Pimentel *et al.*, 2005).
Conventional wheat production usually has higher GWP impact, due to the impact from synthetic nitrogen present in chemical fertilizer. However, adapting organic N inputs to improve crop uptake is a challenge for organic wheat agriculture. Organic remedies, such as organic fertilizers (including manure) and incorporation of nitrogen fixing legume crops instead of chemical fertilizers have been developed to deal with these difficulties while at the same time reducing N losses and subsequent N_2O emissions (Dahlin *et al.*, 2005). Improved fertilizer use efficiency could reduce N_2O emissions from conventional agriculture (Crutzen *et al.*, 2008). In a study by Bloem *et al.*, (1994) about conventional farming performed in the Netherlands, it was concluded that ground water quality was threatened by the use of pesticides for crop protection. With regards to compensating for a lack of chemical pesticides, insect pests and plant pathogens can be effectively controlled in cereal production by employing crop rotations and biological pest managements (Pimentel, 1993).

4.5.3. Social sustainability

Social sustainability of organic wheat farming systems was concluded from the literature to be influenced by farmers' perspectives e.g. motivations or restrictions to practice or ignore organic farming and by agricultural organizations.
Results from questionnaires for the farmers from other studies suggest that the success of sustainable wheat production depends on the motivations, skills, and knowledge of individual farmers. It could be suggested that farmers who are concerned about environmental impacts and healthy food production have higher potential to convert to organic farming system. Thus, an organic farmer could have a lower profit than a conventional counterpart, but his/her main objectives could be met while still having a sufficient income (Nemes, 2009). Additionally, farmers with higher educational background are more motivated to produce organically. So, lack of modern technological knowledge about organic farming systems is a major barrier, as farmers mostly come from uneducated levels of society especially in developing countries as their most important aim is higher yields (Rigby *et al.*, 2001).
So, actions taken by agricultural advisory organizations and local communities are necessary for social sustainability to increase farmers' knowledge on sustainable agriculture practices. Fowler (1999) noted that technical knowledge and management were key issues in best performing farms, and management skills depend on the extension of information and advisory availability and technical support, on the social network of the farmer, and on his/her experience. Thus, it is suggested that advisory organizations promote and motivate conventional farmers by non-economic aspects of organic production which still could meet the main objectives of organic farmers, while having a sufficient income (Lipson, 1999). However, it should be reminded that in some developing countries other problems more than farmers' ideas are barriers for organic

production. For example, in Iran, shortage of water, limited arable land, lack of government support, high soil erosion, and lack of a sound agricultural advisory organization and growing population together, are major barriers that slow down the adoption of sustainable agriculture practices in that country (Chizari *et al*., 2001). If extension service agents want to help conventional wheat farmers to adopt sustainable agricultural practices, they should consider implementing courses identified by organic wheat farmers as important or useful (Chizari *et al.*, 2001).

5. Conclusions

Specific conclusions from this thesis are pertaining to: 1) experimental study of carotenoid content in Swedish adapted organically grown wheat; 2) literature review-based LCA of organic wheat production compared to conventional wheat production; 3) literature review-based farmers'/consumers' perspectives on organic wheat farming.

1. The great variation between the 33 organically grown wheat genotypes in terms of carotenoid contents suggests genetic diversity, which could be exploited in breeding programmes to improve the nutritional quality of organically grown wheat. It may be possible to produce wheat with higher amounts of carotenoids by selecting appropriate wheat genotypes. In doing so, the benefits of organic farming could be expanded to more greatly include positive health benefits. However, carotenoids are not the only healthy compounds that should be considered in the overall picture of nutritional quality of wheat. In future breeding programmes other nutritional elements such as proteins, fibre, vitamins and other healthy compounds should be included in order to breed organic wheat genotypes of high nutritional value. Some genotypes with higher carotenoid amounts from this study that also had relatively higher amounts of tocochromanols from the study of Hussain *et al*. (2012a) were *Olympia*, *Oland 8* and *Aura*, which contained summed amounts of 38.67, 38.63 and 36.6 mg/kg of DW respectively. These genotypes should be of high importance in future breeding programs in terms of nutritional quality.

2. Literature review of LCA was carried out to estimate the agricultural sustainability of organic wheat farming, compared with conventional systems. The conclusions from the literature review can provide a foundation for the inclusion of sustainability methodologies and practices into wheat farming. In terms of local environmental impact, we concluded that organic farming is more sustainable than conventional farming as it relates to soil and water quality. For global impact, it can be said that transport distance is a major determinant for environmental sustainability of organic farming; if organic wheat is produced and sold locally, then environmental impacts would be less in the organic system. However, if transport distances from farm gate to point of sale are large, then there should be no difference in environmental impacts between conventional and organic wheat farming. In addition, organic farming still has not been identified to be 100% economically sustainable, though great progress has been made towards obtaining economic sustainability.

3. Summarised results from other studies indicated that farmers with more educational background and higher economic support were more motivated to perform organic farming than the farmers who were struggling to produce higher yields and were not supported by the organic subsidies. Farmers who valued environmental consideration the most were the most motivated ones to grow organically. It could be concluded that extension organizations and governments are important factors to promote organic farming methods with financial support, while market considerations should not be forgotten as another leading factor to motivate farmers to grow organic.

5.1 Future prospects

5.1.1. Carotenoid compounds in organic wheat

- The promising wheat genotypes (*Olympia*, *Oland 8* and *Aura*) identified based on this study and in the Hussain *et al.* (2012a) study on tocochromanols, are recommended for consideration in organic wheat breeding programs aimed at increasing the nutritional quality of organically grown wheat.
- The genotypes with highest carotenoid content could be of potential interest to be further investigated in products e.g. sprouted wheat, bread etc with respect to consumer preferences.
- This research could serve as a guide for future studies on broader life cycle analyses of organic wheat production of specific genotypes in different farming systems and locations.
- It is recommended that further research could be undertaken in the assessment of farmers' perspectives from several parts of the world (both developed and developing countries) whether it is of interest to grow wheat genotypes with higher carotenoid concentrations.

5.2 Limitations of the study

Interviews with farmers were not actually done using the developed questionnaire. So, the results presented on farmers' opinions are drawn from literature. It would be more precise to interview farmers regarding the selected organic wheat genotypes of this study to gain more accurate results.

Finally, regarding the study of carotenoid amounts, considering the small sample size examined, caution must be applied, as the findings are not transferable to all situations. Larger randomised controlled trials from several parts of the world could provide more definitive evidence to apply for this study. For instance, growing selected organic wheat genotype in Northern countries might not give similar result for other regions with different climate, soil, etc.

6. Acknowledgments

First of all, I would like to thank my father and mother who made these two years of study possible for me, also particular thanks to my life partner, William, who suffered through several grammar checks and reviews of my writing.

Thanks to my supervisor, Ramune Kuktaite for commenting on the thesis and my examiner Eva Johansson. Thank you Maria Luisa Prieto Linde for your valuable efforts on carotenoid analysis and running HPLC. I am also thankful to Lena Ekelund for help with development of the questionnaire and to Abrar Hussain for statistical analysis of the data.

I would also like to thank Christina Kolstrup and Kristina Askard, for their valuable efforts at organizing the Agroecology program and helping with final date management of the thesis.

Thanks to all other professors and lecturers in the Agroecology program, which this thesis was not meaningful without any of them.

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Appendix

Appendix 1. Questionnaire for organic farmers

General questions

1. Give a short summary of your farming history: how long have you been an organic farmer? Why did you become an organic farmer? Are you satisfied with the organic farming method?

2. What wheat variety do you grow? Which are the most important factors for choice of variety?

3. How do you see your farming business in future? Do you foresee any problems related to your farming practices? What solutions could be implemented to manage those problems?

Quality

4. How do you define high quality wheat?

(a. wheat with more healthy compounds b. better colour c. larger seeds d. other).

5. Is there a trade-off between high yield and better soil quality?

6. What are the advantages of organic farming? Consider the following:

(a. wheat with more healthy compounds b. better taste c. market demand d. better soil quality e. Reduced environmental pollutions g. no chemicals in wheat f. Other reasons).

7. What are the disadvantages of organic farming? Consider the following:

(a. Low yield b. less economical benefits c. less market demand d. higher input prices e. higher selling prices in the market f. Other reasons).

Health aspect

8. What is your idea about chemical fertilizers, pesticides or herbicides in agricultural products?

9. How healthy is organic food to compare with conventional?

(a. Less healthy b. Healthier c. No idea).

10. How interested are you in growing wheat varieties with higher carotenoid amount? (Explain benefits of carotenoids to farmers)

(a. Very interested b. Not interested c. No idea). Give reasons for responses.

Business and management

11. Are you satisfied with the current marketing system/ value chain? Would you like to change anything? Who are your buyer/buyers?

11. Do you find it easy to find enough buyers/ customers?

12. How does your market evaluate better quality? Are you paid for better quality?

13. How much are you satisfied with the income? (a. 100% b. 50% c. 0%). Give reasons for responses.

14. To what degree are you satisfied with yield? (a.very satisfied b. Reasonable yield c. Not satisfied).

15. To what degree are you satisfied with the profits? (a.very satisfied b. Reasonable income c. Not satisfied).

15a. Would you prefer having higher profits to the current situation?

Environmental questions

16. How do you evaluate your farm land and soil quality to compare with conventional farming? (a. High quality b. Low quality c. No idea). Give reasons for responses.

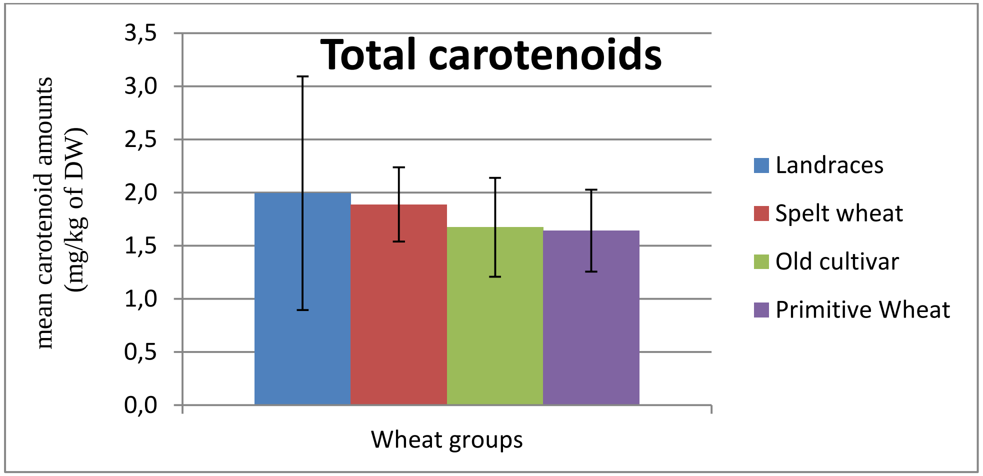
17. How do you evaluate the water quality of your farmland? (a. High quality b. Low quality c. No idea).

Education and information questions

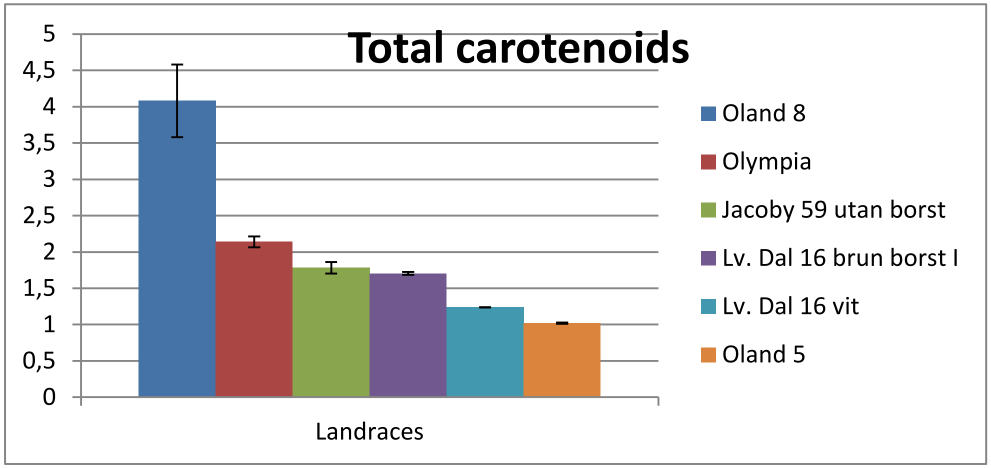
18. How did you learn to implement organic farming practices?

19. Do you receive enough professional advice regarding technical problems and support from advisory consultants? (a. Yes b. No).

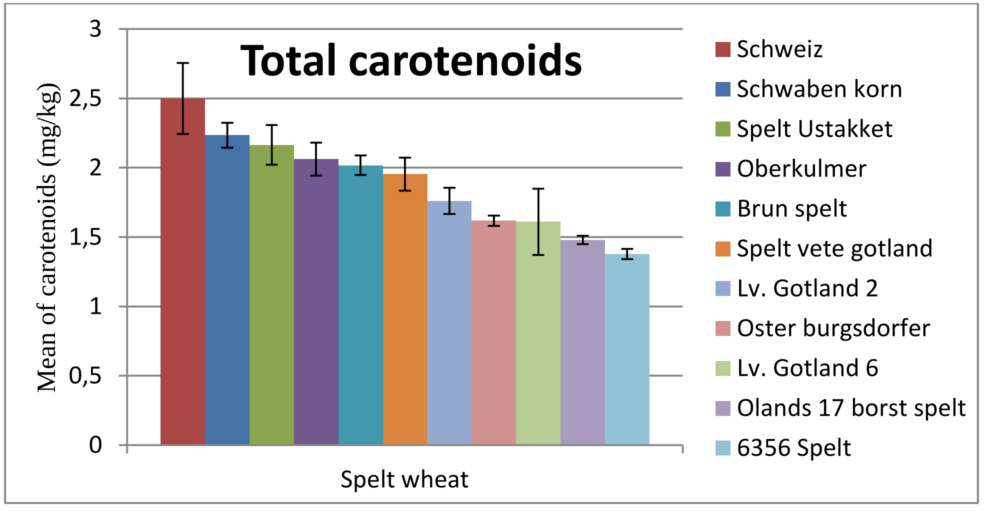
Appendix 2. Total amount of carotenoids within wheat groups. Error bars show standard deviation.



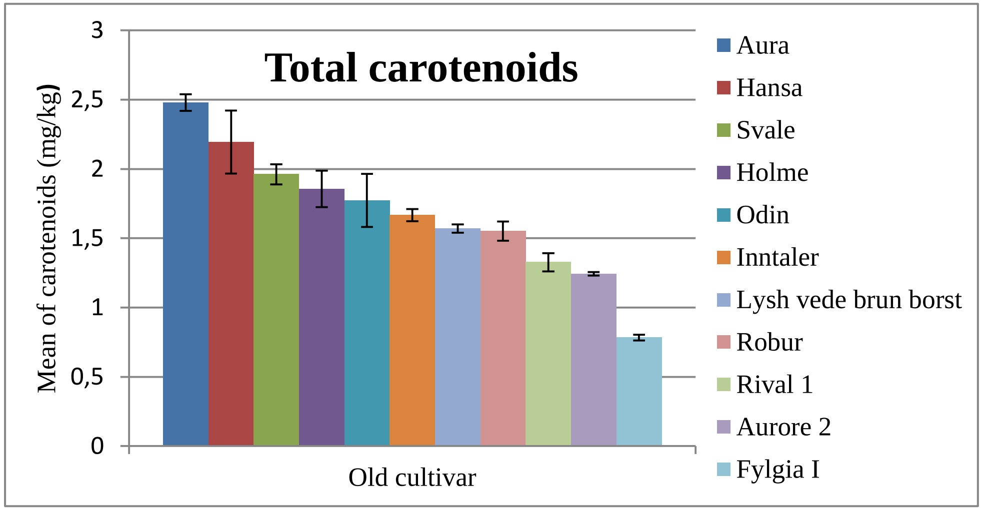
Appendix 3. Total carotenoid contents for landraces. Error bars show standard deviation.



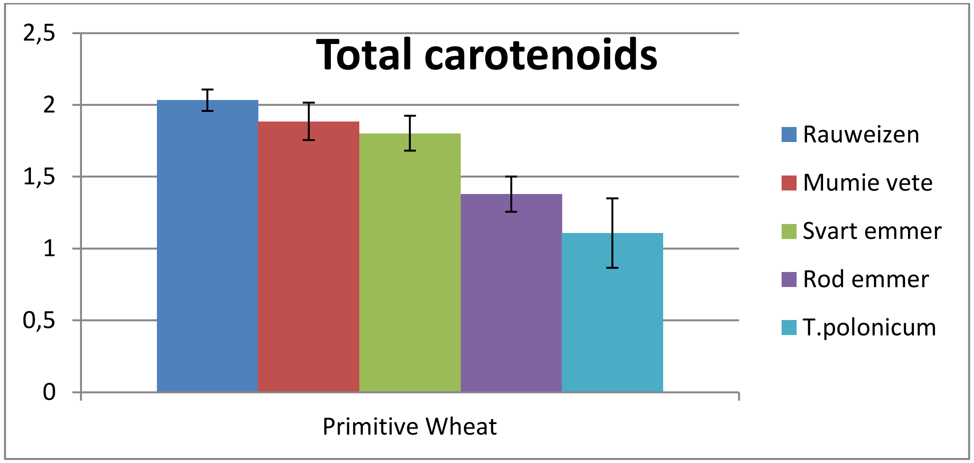
Appendix 4. Total carotenoid contents for spelt wheat. Error bars show standard deviation.



Appendix 5. Total carotenoid contents for old cultivars. Error bars show standard deviation.



Appendix 6. Total carotenoid contents for primitive wheat varieties. Error bars show standard deviation.



Appendix 7. Carotenoid amounts between 33 organic wheat genotypes.

Sr_No	Genotype	Group	Type	β-carotene	β-cryptoxanthin	Lutein	Zeaxantin	Total
47	Oland 8	Landraces	S	0.23 (c)	0.01 (b)	3.70(a)	0.13(g)	4.08(a)
40	Schweiz	Spelt wheat	W	0.20 (de)	0.007 (de)	2.21(b)	0.07(h)	2.49(b)
48	Aura	Old Cultivar	W	0.28 (a)	0.01 (a)	2.04(bc)	0.13(g)	2.47(b)
45	Schwaben korn	Spelt wheat	W	0.16 (fg)	0.005 (hij)	1.97(c)	0.09(h)	2.23(c)
4	Hansa	Old Cultivar	W	0.03 (mnop)	0.0007(mno)	1.98(c)	0.17(cd)	2.19(cd)
44	Spelt Ustakket	Spelt wheat	W	0.12 (ijk)	0.003 (k)	1.90(cd)	0.13(g)	2.16(cde)
9	Olympia	Landraces	W	0.02 (op)	0.0005 (no)	1.97(c)	0.14(fg)	2.13(cdef)
43	Oberkulmer	Spelt wheat	W	0.09 (l)	0.003 (k)	1.86(cde)	0.09(h)	2.06(cdefg)
10	Rauweizen	Primitive wheat	W	0.02 (p)	0 (o)	1.76(def)	0.24(a)	2.03(cdefg)
41	Brun spelt	Spelt wheat	W	0.20 (d)	0.008(d)	1.70(ef)	0.09(h)	2.01(defg)
13	Svale	Old Cultivar	W	0.02 (op)	0.0002(o)	1.76(def)	0.17(cd)	1.96(efgh)

29	Spelt vete gotland	Spelt wheat	W	0.18 (ef)	0.007(d)	1.61(fg)	0.14(efg)	1.95(fgh)
49	Mumie vete	Primitive wheat	W	0.01 (p)	0.0003(o)	1.73(def)	0.14(g)	1.88(ghi)
39	Holme	Old Cultivar	W	0.05 (m)	0.002(l)	1.60(fg)	0.19(bc)	1.85(ghij)
30	Svart emmer	Primitive wheat	W	0.03 (mnop)	0.001(mn)	1.61(fg)	0.15(defg)	1.80(hijk)
6	Jacoby 59 utan borst	Landraces	W	0.26 (b)	0.009(c)	1.37(hij)	0.13(g)	1.78(hijk)
8	Odin	Old Cultivar	W	0.10 (l)	0.005(hij)	1.49(gh)	0.17(cd)	1.77(hijkl)
27	Lv. Gotland 2	Spelt wheat	S	0.09 (l)	0.004(k)	1.39(hij)	0.26(a)	1.76(hijkl)
25	Lv. Dal 16 brun borst I	Landraces	S	0.02 (nop)	0.0008(mno)	1.51(gh)	0.15(defg)	1.70(ijklm)
5	Inntaler	Old Cultivar	W	0.04 (mno)	0.001(m)	1.48(ghi)	0.13(g)	1.66(jklmn)
42	Oster burgsdorfer	Spelt wheat	W	0.10 (kl)	0.006(fg)	1.35(hij)	0.15(defg)	1.61(klmn)
46	Lv. Gotland 6	Spelt wheat	S	0.12 (ij)	0.006(fg)	1.30(ijk)	0.17(cde)	1.61(klmn)
7	Lysh vede brun borst	Old Cultivar	W	0.11 (ijkl)	0.005(ij)	1.23(jklm)	0.21(b)	1.57(mno)
12	Robur	Old Cultivar	W	0.13 (hi)	0.005(ij)	1.25(jkl)	0.15(defg)	1.55(mno)

18	Olands 17 borst spelt	Spelt wheat	S	0.13 (hi)	0.006(gh)	1.14(klmn)	0.19(bc)	1.47(nop)
31	Rod emmer	Primitive wheat	W	0.01 (p)	0.0003(o)	1.10(lmno)	0.25(a)	1.37(opq)
1	6356 Spelt	Spelt wheat	W	0.15 (gh)	0.007(ef)	1.05(mnop)	0.16(defg)	1.37(opq)
21	Rival 1	Old Cultivar	S	0.10 (jkl)	0.004(jk)	0.95(op)	0.26(a)	1.32(pq)
17	Aurore 2	Old Cultivar	S	0.13 (i)	0.006(fg)	1.01(nop)	0.09(h)	1.24(qr)
19	Lv. Dal 16 vit	Landraces	S	0.13 (ghi)	0.005(ghi)	0.88(pq)	0.20(b)	1.23(qr)
33	T.polonicum	Primitive wheat	W	0.04 (mn)	0.001(m)	0.88(pq)	0.17(cdef)	1.10(rs)
24	Oland 5	Landraces	S	0.03 (mnop)	0.001(mn)	0.71(qr)	0.26(a)	1.01(s)
23	Fylgia I	Old Cultivar	S	0.02 (nop)	0.001(m)	0.60(r)	0.15(defg)	0.78(t)

Means with the same letter are not significantly different. (LSD=0.2074 mg/ kg of DW). W=winter wheat, S=spring wheat.

Appendix 8. Differences of mean carotenoid amounts within 33 organic wheat genotypes

	β-carotene	β-cryptoxanthin	Lutein	Zeaxanthin	Total
Mean of carotenoids	0.11	0.005	1.52	0.17	1.80
LSD	0.02	0.0009	0.19	0.030	0.21
Mean-LSD	0.08	0.004	1.34	0.14	1.59
P-value	<.0001	<.0001	<.0001	<.0001	<.0001

Significant difference: Mean-LSD≥ LSD and p-value<0.05.

Appendix 9. Most important reasons for farmers to terminate their organic licence **Adapted from *Rigby et al., 2001.***