Quality comparison of organic and conventional wheat by the use of common and holistic methods of analysis

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All truths are easy to understand
once they are discovered.
The point is to discover them.

Galileo Galilei

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Summary

The market for organic food is strongly growing. There are many reasons why consumers buy products from organic agriculture. Some reasons are attributed to the product quality as well as to the assumption about the production process used in organic agriculture. Organic food stands for a sustainable and socially acceptable production. In addition, the desire for a healthy nutrition is important. So in summary consumers chose organic products because they trust in a number of attributes related to the product itself and it’s production process.

During the last years, food scandals developed in conventional agriculture as well as in organic agriculture. Therefore the consumers lost some of their confidence in organic products. With a growing production the risk for more scandals might be rising. Thus emerged a need for methods, which can examine the quality of organically produced food (authenticity examination) and compare it with the quality of conventional products.

In such comparison studies it is of particular importance to use a possibly wide range of analysis methods, because the quality differences due to the farming system can appear in crops in various forms – in the contents of singular compounds, as well as in the structural features, which only can be analyzed on the whole product (Kokornaczyk et al., 2008). Therefore, the common and holistic methods of analysis have been united in the current research in order to receive complementary information about the given samples.

The aim of the research was to examine and compare the quality of the organically and conventionally grown grain samples. The samples derived from the Mediterranean Arable System Comparison Trial (MASCOT) in Italian Toscana, a long-term experiment launched in 2001 and carried out at the Interdepartmental Centre for Agri-environmental Research “E. Avanzi” (CIRAA) of the University of Pisa.

The choice of the applied analysis methods was based on the founds available in literature. The organically and conventionally grown wheat often differs in total protein content, which depends on the availability of N and is also an important indicator of the technological quality of grain (Alföldi et al., 2006). The content of carotenoids, which belong to the secondary plant metabolites, was found to differ in organic and conventional crops because of the different growth conditions, like exposure to pests

and diseases (Alföldi et al., 2006). Many studies were conducted on the antioxidants contents present in different wheat varieties (Zhou et al., 2004; Adom et al., 2003). But to our knowledge there is still limited literature on the contents of antioxidants in wheat samples deriving from differently managed farming systems. Studies indicate that holistic methods, such as biocrystallization, are especially suitable for authenticity tests of organic produce, hence a validation of these methods has been demanded (Siderer et al., 2005).

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1. INTRODUCTION

1.1. Organic agriculture

The organic agriculture is defined as an agricultural system, where the use of synthetic pesticides and fertilizers is limited to almost nothing (Council Regulations, 1991).

The idea is to utilize the ecological mechanisms operating in nature. Plant nutrients are supplied through crop rotation and manure from farm animals in appropriate numbers. Plants are protected by the use of resistant varieties and a number of management tools, to avoid, rather than combat, problems with weeds, pests and diseases (Le Guillou et al., 2001).

This management was the generally used technology before the invention of artificial fertilizers and pesticides. In the traditional agriculture, in pre-industrial society, yield was generally limited by the low availability of plant nutrients, just like in most of nature, and the natural defence mechanisms of the plant were fully operative.

Throughout the 1950s, the main aim of farming was to achieve a major improvement in productivity so as to satisfy immediate needs for food and raise the European Community's rate of self-sufficiency. In these circumstances, organic farming was obviously unlikely to be viewed very favourably (Le Guillou et al., 2001).

Through many years in the western society, there were people who insisted on the use of organic agriculture, but they were minorities, often connected to particular believes or religions. However with the increased emphasis on pollution and other negative effects of technology, society began limiting the use of most dangerous pesticides. One of the methods was the promotion of organic agriculture in order to protect against pollution (Brandt et al., 2001). Organic food became established as a separate identity in the public's mind during the 1960s and 1970s (Baker, 2005).

1.1.1. Definition of organic agriculture

In accordance with the "Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods" of Codex Alimentarius (2001) the organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity. It emphasizes the use of management practices to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, cultural, biological and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system.

The Codex Alimentarius guidelines (2001) specify that an organic production system is designed to:

a. “enhance biological diversity within the whole system;
b. increase soil biological activity;
c. maintain long-term soil fertility;
d. recycle wastes of plant and animal origin in order to return nutrients to the land, thus minimizing the use of non-renewable resources;
e. rely on renewable resources in locally organized agricultural systems;
f. promote the healthy use of soil, water and air as well as minimize all forms of pollution thereto that may result from agricultural practices;
g. handle agricultural products with emphasis on careful processing methods in order to maintain the organic integrity and vital qualities of the product in all stages;
h. become established on any existing farm through a period of conversion, the appropriate length of which is determined by side-specific factors such as the history of the land, and type of crops and livestock to be produced.”

1.1.2. Principles of organic agriculture

“The International Federation of Organic Agriculture Movements (IFOAM) is a worldwide operating organization, whose goal is to lead, unite and assist the organic movement in its full diversity. IFOAM’s goal is the worldwide adoption of ecologically, socially and economically sound systems that are based on the principles of Organic Agriculture.”

The principles of organic agriculture are IFOAM’s answer on the continuous growth of the organic sector and on the challenges and opportunities that came with that growth. In 2003 a process started, in which IFOAM was engaged in the articulation of Principles of Organic Agriculture with the aim to create basic values, the fundamental underpinning for organic agriculture. This process ended in September 2005, on the Federation’s General Assembly. The resulting principles serve to both inspire the organic movement in its full diversity, and to articulate the meaning of Organic Agriculture to the world at large (Luttikholt et al., 2006).

The four IFOAM principles:

- the principle of health,
- the principle of ecology,
- the principle of fairness and
- the principle of care

are seen as roots, from which the organic agriculture grows and develops.

Principle of health

Organic Agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible.

This principle points out that the health of individuals and communities cannot be separated from the health of ecosystems - healthy soils produce healthy crops that foster the health of animals and people.

Health is the wholeness and integrity of living systems. It is not simply the absence of illness, but the maintenance of physical, mental, social and ecological well-being. Immunity, resilience and regeneration are key characteristics of health.

The role of organic agriculture, whether in farming, processing, distribution or consumption, is to sustain and enhance the health of ecosystems and organisms from the smallest in the soil to human beings. In particular, organic agriculture is intended to produce high quality, nutritious food that contributes to preventive health care and well-being. In view of this it should avoid the use of fertilizers, pesticides, animal drugs and food additives that may have adverse health effects (IFOAM, 2008).

Principle of ecology

Organic Agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.

This principle roots organic agriculture within living ecological systems. It states that production is to be based on ecological processes and recycling. Nourishment and well-being are achieved through the ecology of the specific production environment.

For example, in the case of crops it is the living soil; for animals it is the farm ecosystem; for fish and marine organisms, the aquatic environment.

Organic farming, pastoral and wild harvest systems should fit the cycles and ecological balances in nature. These cycles are universal but their operation is site-specific. Organic management must be adapted to local conditions, ecology, culture and scale. Inputs should be reduced by reuse, recycling and efficient management of materials and energy in order to maintain and improve environmental quality and conserve resources.

Organic agriculture should attain ecological balance through the design of farming systems, establishment of habitats and maintenance of genetic and agricultural diversity. Those who produce, process, trade, or consume organic products should protect and benefit the common environment including landscapes, climate, habitats, biodiversity, air and water (IFOAM, 2008).

Principle of fairness

Organic Agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities

Fairness is characterized by equity, respect, justice and stewardship of the shared world, both among people and in their relations to other living beings.

This principle emphasizes that those involved in organic agriculture should conduct human relationships in a manner that ensures fairness at all levels and to all parties - farmers, workers, processors, distributors, traders and consumers. Organic agriculture should provide everyone involved with a good quality of life, and contribute to food sovereignty and reduction of poverty. It aims to produce a sufficient supply of good quality food as well as other products.

This principle insists that animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behaviour and well-being.

Natural and environmental resources that are used for production and consumption should be managed in a way that is socially and ecologically just and ought be held in trust for future generations. Fairness requires systems of production, distribution and trade that are open, equitable and account for real environmental and social costs (IFOAM, 2008).

Principle of care

Organic Agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

Organic agriculture is a living and dynamic system that responds to internal and external demands and conditions. Practitioners of organic agriculture can enhance efficiency and increase productivity, but this should not be done at the risk of jeopardizing health and well-being. Consequently, new technologies need to be assessed and existing methods reviewed. Given the incomplete understanding of ecosystems and agriculture, care must be taken.

This principle states that precaution and responsibility are the key concerns in management, development and technology choices in organic agriculture. Science is necessary to ensure that organic agriculture is healthy, safe and ecologically sound. However, scientific knowledge alone is not sufficient. Practical experience, accumulated wisdom and traditional and indigenous knowledge offer valid solutions, tested by time. Organic agriculture should prevent significant risks by adopting appropriate technologies and rejecting unpredictable ones, such as genetic engineering. Decisions should reflect the values and needs of all who might be affected, through transparent and participatory processes (IFOAM, 2008).

1.1.3. IFOAM’s basic standards regarding the organic agriculture and food processing

In 1972 the International Federation of Organic Agriculture Movements (IFOAM) has been formed, an international umbrella association for the organic agriculture. In 1980 this organisation has for the first time established generally binding standards for the organic agriculture and organic food processing. The IFOAM’s Basic Standards are divided into General Principles, Recommendations, Basic Standards and Derogations (IFOAM, 2002).

In part 6, Processing and Handling IFOAM defines:

General Principle
Organic processing and handling provides consumers with nutritious, high quality supplies of organic products and organic farmers with market without compromise to the integrity of their products.

In point 6.1.2 is specified:
All organic products shall be clearly identified as such, and stored and transported in a way that prevents contact with conventional product through the entire process.

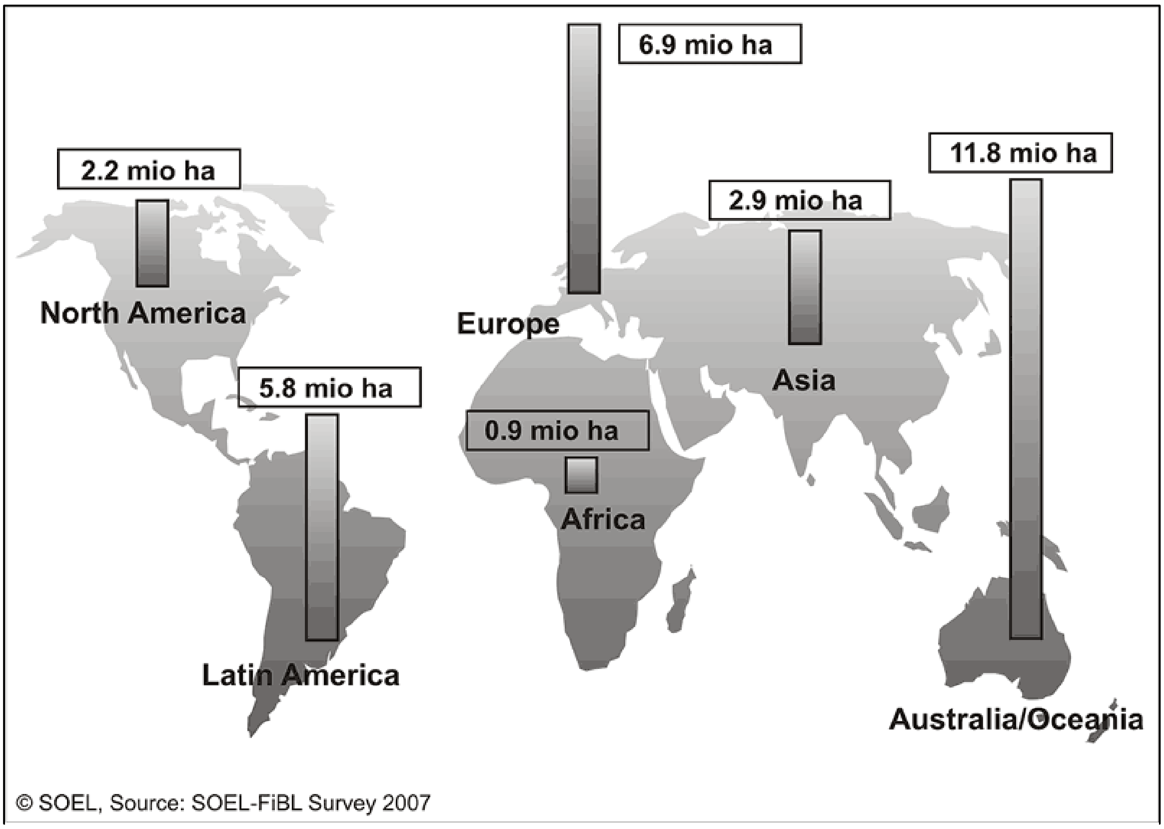
Point 7 a contains specifications to the labelling of organic products:
Organic Products are clearly and accurately labelled as organic.

Point 6.3 states a general principle
Organic food is processed by biological, mechanical and physical methods in a way that maintains the vital quality of each ingredient and the finished product.

Kahl (2006b) concludes the IFOAM quality recommendations: Authentic products of high quality are produced, whose processing should maintain the product’s vital quality. However there is still investigation needed on how can “high quality” be defined, and the authenticity and vital quality of organic products verified.

1.1.4. Development of organic agriculture worldwide

Recently the demand for organically produced goods is rapidly growing. The market for organic foods increased during the last 10 years and today this market share has been showing the highest level of growth of all food sectors (Meier-Ploeger, 2005a). At the same time the organically cultivated land areas are continuously increasing. The amount of organic land areas worldwide is presented below.



Graphic 1: Development of organic agriculture worldwide.

The development of organic agriculture is rapid - organic farming is currently practiced in more than 120 counties around the world, on an area of almost 31 million hectares and is managed by at least 633.891 farmers. This constitutes ca. 0.7 percent of the agricultural land of the countries covered by the survey. The continents with the largest organic land areas are: Oceania, with 39 percent of the world’s organic land, followed by Europe (23 percent) and Latin America (19 percent). While the leading countries are: Australia (11.8 million hectares), Argentina (3.1 million hectares), China (2,3 million hectares) and the US (1,6 million hectares). However the number of farms and the proportion of organically compared to conventionally managed land is highest in Europe. Recently the major growth of organic land has been evident with both North America and Europe each showing an increase of half a million hectares at the end of 2004. (Willer et al., 2007).

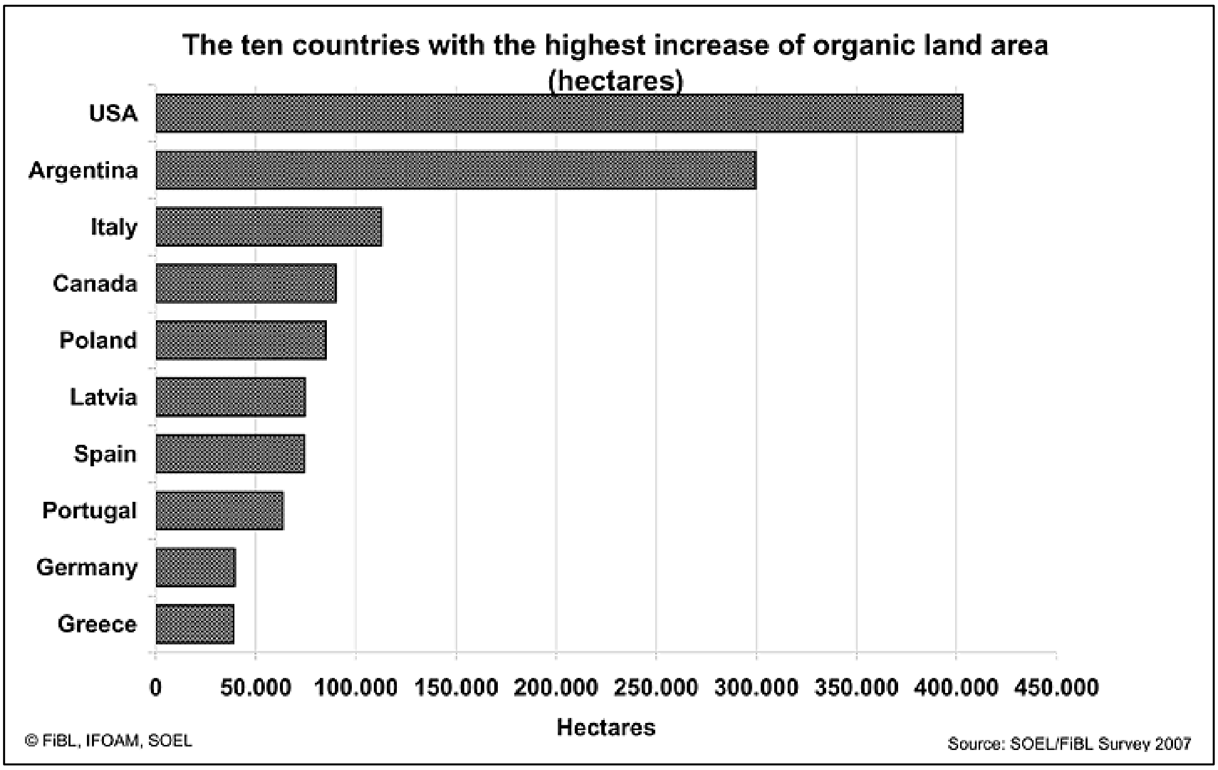
Considering the data from the study ‘Overview of production and marketing of organic wild products’, carried out by the International Trade Centre ITC and the Organic Services in 2005, there are about 62 million hectares of organic wild collection, and a number of 979 organic wild collection projects. Europe and Africa have the largest collection areas (almost 27 million hectares each).

According to the data about the main land uses and the importance of some crops in the global context, Italy is the world's leader in the organic production of citrus fruits; Mexico is the biggest organic coffee producer and the Dominican Republic is the major producer of organic cocoa. The leaders in organic grape production are Italy, Spain and France and the largest areas of organic olives are in Italy, Spain and Tunisia.

1.1.5. Organic agriculture in Europe

Since the beginning of the 1990s, organic framing has quickly developed in almost all European countries. In the end of 2005 6,9 hectares were managed organically in Europe, from which 6,3 hectares inside the European Union. From all European Countries Italy has the largest area of organically managed land and the highest number of organic farms.

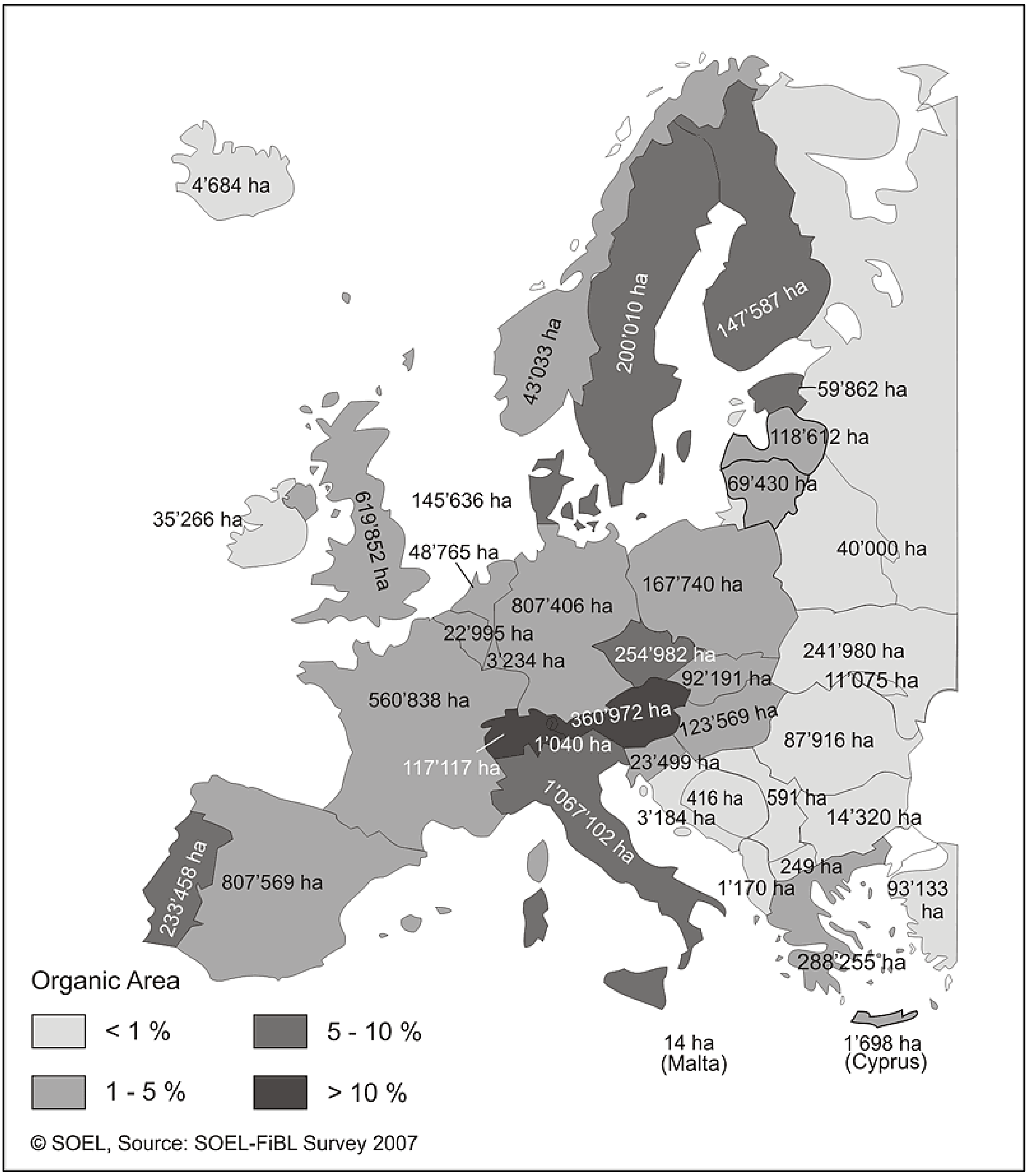
Since 2004 the area of organic land in Europe increased by 8 percent (by almost 510.000 hectares) and inside the European Union by 8.5 percent (490.000 hectares). The increase in the EU is due to the high growth rate in the new member states (for instance Lithuania and Poland) and the substantial increases in Italy and Spain. The European market is estimated to be between 13 and 14 billion Euros (2005). The growth of the market compared to the previous year is about 10 percent. Germany is the biggest market for organic products with an annual turnover of 3.9 billion Euros, followed by Italy (2.4 billion Euros) and France (2.2 billion Euros). The highest share of organic products of the total market is Switzerland with 4.5 percent, and the highest per capita consumption is also in that country with more than 100 Euros spent on organic food per year and citizen.



Graphic 2: The ten countries with the highest increase of organic land area.

Global sales of organic food and drink have increased by 43 percent from 23 billion US-Dollars (17.8 billion Euros) in 2002 with sales reaching 33 billion US-Dollars (25.5 billion Euros) in 2005. Organic Monitor expected sales to come near 40 billion US-Dollars (30.9 billion Euros) in 2006. Although organic agriculture is present now in most parts of the globe, the demand remains concentrated in Europe and North America. These regions are experiencing undersupply because production is not meeting demand and thus have large income of imports (Yussefi et al., 2007).

In Europe, the country with the biggest amount of organic land is Italy with 1.067.102 hectares of organically managed areas. It is also the country with the biggest increase of organic land area.



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Graphic 3: The amount of organic land area within the European counties.

The economic value of the Italian organic market is estimated to be around 1.45billion Euro and the growth trend is very strong.

Food scares about mad cow disease led to an acceleration of the growth: in the first three months of 2001, the greatest wholesalers reported a growth in sales volume from 40 to 65 percent in respect to the same months of 2000. It is therefore no surprise that the big national companies and the Italian subsidiaries of multinational corporations have shown great interest in organic agriculture and food trade, launching new lines of organic products or taking over businesses operating in this sector (Yussefi et al., 2007).

1.2. Food quality

The food quality is a very wide topic, which can be seen and discussed from many different points of view, in dependence on the interests of all - involved people within the chain of food production, food processing and consumption. Food quality is also influenced by cultural habitudes and religion. It is a meeting point of several symbolic codes: personal, familiar, cultural, biological, industrial and environmental, as well as ethical dimensions of social justice (James, 1993)

1.2.1. Food quality in history

Centuries ago the purpose of farming was the production of food for the population of the country. The region, especially the capacity of the soil and climatic conditions, influenced the agro-biodiversity and the amount of what could be planted and harvested. Hilly areas were regions for raising sheep, goats and cows and special breeds were selected according to vegetation and the climatic conditions. Typical landscapes, reflecting the agricultural practice, developed over centuries (Fernandez-Armesto, 2001). The Peoples diets differed between the different regions of a country, based on the treasures of farm products within the different seasons. Each region had its typical recipes, which were developed in households and small scale processing companies and varied in accordance to the season. To preserve seasonal products different processing techniques had been developed (preservation with sugar, salt, vinegar, fermentation, heat, drying) (Montanari, 1993; Braudel, 1985). There was a natural understanding of the rhythm of nature, agriculture, food and landscape and a deep trust in good food for a good health (Conford, 2001).

In the 19th century the industrialisation process also influenced the food market. Because of the separation of home and the working place in bigger cities, the need for convenience products occurred. Prepared soups and breakfast cereals were the first products introduced to a growing market. The regional recipes have been replaced by new “global” recipes and the consume of frozen food and food derived from heated glass houses caused an independence of peoples food habits from the seasonal conditions. Food processing became a more frequent practice at both European and international level. The advertisement: “You can rely on us – it tastes always the same” expressed this global and unseasonable demand. The trust in a farmer and his/her products changed towards trust in a company and its Quality Management System (QM) as well as the trust in legislative requirements for a healthy food from government (CONFORD, 2001). The new generation of convenience products in the global market caused not only the independence of food from region and season but also from agricultural products. The so called food design technique allows companies to purchase the cheapest carbohydrates (extracted from yams or potatoes e.g. as a basis for a soup), fats or proteins and to “fortify” these ingredients with vitamins, minerals, flavour, colouring additives, processing auxiliaries. The role of row material and its structure became less important. It can be sad, that food today is independent from region, season and agriculture (Meier-Ploeger, 2005b)

1.2.2. Product related and process related quality of foods

The quality of foods depends on both the quality of the primary product as it’s processing. The product related quality arises during growth and differentiation of the crop (Bloksma et al., 2003) and thus is influenced by the growing conditions and the environment. The process related quality depends on food processing and the control systems applied and regards the maintenance and conservation of the natural product quality, but not its creation, like for instance the system of Hazard Analysis and Critical Control Point – HACCP (Bryan, 1990),

The product related quality is strictly associated with the quality of soil, the state of the environment and applied agricultural management.

1.2.3. Organic food quality

It is well known that the agricultural system influences the quality of crops and produced foods (Heaton, 2001). In particularly it can influence the food quality in terms of safety, nutritional content, biological value, and sensorial characteristics where:

- food safety indicates the extent, to which the foods contain undesirable components such as potentially harmful pesticide residues, food additives and pathogens.
- nutritional content regards the food’s content of substances to be known to contribute to good health like vitamins, minerals, secondary plant metabolites and others.
- nutritional value describes the capacity of a food to support health, growth, and reproduction (Heaton, 2002).
- and sensorial characteristics involve food’s features like taste, smell, and consistence (Burgt, 2005).

In addition to this standard approaches to organic food quality some researchers claim that the view on food quality should be complemented by more elements and aspects, which are vital for health. This extended approach to food quality, known as the holistic approach, understands crops and foods as living substances and assumes that they can differ not only in the contents of their ingredients but also in their “vitality” or “organising activity” – features, which can not be assessed by the commune methods of analysis (Balzer-Graf, 2000).

1.2.4. Consumers perception of organic food in Europe

The consumers understanding of what food quality means is related to many factors and in accordance to the holistic approach to food quality suggested by Meier-Ploeger and Vogtman (1991), it relays on social, psychological, environmental and political dimensions. For this reason the main outlines of consumers’ perception of organic food can differ from country to country.

The European market of organic foods and drinks is a big and dynamically developing market. The European consumer perception of organic food is therefore an important and interesting topic.

A study designed by O’Doherty et al. (2004) compares the perceptions of organic foods, which are fundamental for the buying motives of European consumers coming from Denmark, Great Britain, Italy and Hungary.

The Danish consumers concern the following parameters as important in their assessment of organic and conventional foods: regarding the eating quality consumers indicated that some organic products have a better taste, than their conventional counterparts. Regarding other quality aspects, importance is attributed to effects on production and processing on the environment, animal welfare, human health and life quality, as well as the production and sale of foods within the domestic market, as contrasted with imported products. In regard to safety, fear and worry are expressed about the use of chemical pesticides, medicines, growth hormones in animal production, food pathogens of significance for human health and the possibility of GM contamination of organic products.

<u>British consumers</u> identify organic products with a better taste or they associate organic food with a quality of "home-made" food. They perceive organic food as beneficial for health, safety, environmental soundness and other related issues. British consumers see the organic food as "pure" and " natural", free from pesticides, artificial additives and fertilizers. A small survey conducted in the Newcastle area indicates that "natural", "not intensive", production "without growth hormones" are key element in consumers' interpretation of the term "organic farming" (Hutchins and Greenhalgh 1997). The British consumerism has also a strong moral dimension, which is named as the "new wave", characterized by the connections between production and consumption, both at local and global levels, where issues such as fair trade, workers social rights and environmental impacts in the third world producer countries are central concerns, for example for the environment, animal welfare, equity among people involved in the food chain or who are affected by the use of natural resources, social rights for workers, care for health of people you serve food etc.

British consumers are also concerned about the avoiding in organic production of unnecessary food-miles, packing, and in general excessive use of energy in all levels of the food chain.

For the <u>Italian consumers</u> appearance and taste are reported to be of importance for choosing organic products. However, other studies indicate that Italian consumers do not seem to prioritise the appearance of products, thus the indication that they have other quality measurements apart from appearance when it comes to evaluating food. For the majority of Italian consumers health is a very important issue, and it might be even more prominent than the environmental issue in Italians' self perception of their reasons for buying organic food. Consumers who buy organic foods seem, in general, to be more ethically concerned and idealistic than conventional consumers. For Italian people the origin of food is of great importance, but one study indicates that consumers view origin of the food as a proxy for quality. With regard to safety, worry is expressed in regard mainly to the use of chemical pesticides in agricultural production.

With regard to quality aspects emphasizes by Hungarian consumers buying organic food Health is reported to be of main importance. There is, moreover, reference to a general view that agriculture is not associated with environmental pollution (Fruehwald, 2000; Kuerhy-Baricz, 1996).

1.2.5. Organic and conventional produced foods in comparison

People's health is influenced by many factors, like the presence of environmental pollutants, their genetic make-up and lifestyle aspects such as smoking, lack of exercise, excessive alcohol consumption, effects of drugs and emotional distress, to list a few.Diet is known to play a key role in between of all these factors.

The link between dietary choices and the occurrence of diseases in humans is clear. Recently worldwide many government and non-government institutions are occupied with the promotion of a healthy and balanced diet, with the scope of reducing the incidence of chronic diseases. The Italian Ministry of Health is carrying into effect a program for the diffusion and development of a "correct culture of alimentation" *(diffondere e sviluppare una "cultura corretta alimenta-zione")*. The main goal of this program is to reduce the incidences of obesity and other chronic diseases in the Italian population, especially for children (Furiozzi, 2002). The British Health Education Authority promotes a major consumption of fruits and vegetables (five times a day) and a decreased consumption of fat to lower one's risk of cancer and hart diseases (Health Education Authority, 1995).

It is well known, that food compounds, such as phytochemicals (every naturally occurring chemical substance present in plants, especially those that are biologically active) reduce the risk of cancer and cardiovascular diseases (Yu et al., 2002) and the intake of fiber reduces the occurrence of diabetes, coronary heart disease and intestinal cancer (Adom et al., 2003). But not only the dietary choices are important, the quality of the chosen products also counts.

The organic agriculture is capable to increase the quality of produced foods in terms of food safety and nutrient composition.

In his review of evidence “Organic Farming Food Quality and Human Health”, where over 400 published papers considering or comparing organic and non-organic foods were examined, Heaton (2001) expresses the opinion that “eating organically grown food is likely to improve one’s intake of minerals, vitamin C and antioxidant secondary nutrients while reducing exposure to potentially harmful pesticide residues, nitrates, GMOs and artificial additives used in food processing”.

According to Heaton (2001) when reducing the concept of food quality to the absence of toxins and presence of primary and secondary nutrients, it can be asserted, that the organically grown products:

- are more safe than the non-organic products, because of the techniques used in the organically managed farming system. Eating organic products minimizes the exposure to residues of pesticides, antibiotics and high nitrate contents, eliminates the exposure to a large range of food additives, and the risk of exposure to Bovine Spongiform Encephalophaty (BSE).
- contain more primary nutrients, such as vitamins, minerals and dry matter.
- contain more antioxidant secondary nutrients (Heaton, 2001).

Table 1 shows a comparison of results from seven literature reviews on the quality of organic and conventional foods. According to this table organic products tend to have a slight greater content of minerals, vitamins, and plant secondary nutrients. The protein quality of these products is slightly better than the protein quality of conventional products. Furthermore the organic products score higher in terms of nitrate content, and the content of pesticide residues. The organic products tend to have a slightly lower content of proteins, what, in regards to wheat, results in a slightly worse baking quality. At the same moment the enjoyment of eating the organic products is slightly better in comparison to the conventional products. There has been no difference found in the contents of heavy metals and the contamination by pathogenic microorganisms of organic and conventional products (Alföldi, 2006).

	Woese et al., 1995	Worthin-gton, 1998	Heaton, 2001	Bourn & Prescott, 2002	Levimirov & Müller, 2003	Tausch-er et al., 2003	Afssa, 2003	**Overall trend**
Quality in terms of nutritional physiology								
Desiderable substances								
Minerals	=	+	+		++	++	++	**+**
Protein content	-	-	-	-	-	-	-	**-**
Protein quality		+			+		+	**+**
Vitamins	+	+	+		++	+	+	**+**
Plant secondary metabolites			+		++	++	+	**+**
Undesiderable substances								
Nitrate	++	++	++	+	++	++	++	**++**
Pesticide residues	++		++	++	+	++	++	**++**
Pathogenic micro-organisms			=	=		=		**=**
Heavy metals	=	+	=			++	=	**=**
Suitability								
Suitability for baking - wheat	- -		-		- -	-		**-**
Sensory Quality								
Enjoyment	+		+		++	+		**+**

Table 1: Results of seven literature reviews carried out between 1995 and 2003 comparing organically and conventionally grown products, where: (++) organic products score higher than conventional products,(=) organic products have a slight advantage, (=) no difference, (-) organic products have a slight disadvantage, (- -) organic products score less well than conventional products, ( ) no comment made or no general conclusions drawn (Alföldi, 2006).

1.2.6. How organic farming influences the food quality

The cropping system influences the foods quality mainly through the impact on the soil, it’s characteristics, nutrients, and microorganisms.

According to Sir Albert Howard (1873-1947), a British botanist and a pioneer of organic farming: “The birthright of all living things is health.” This is true for soil, plant, animal and man, i.e. the health of each is connected like links of a chain. Any weakness or defect in the health of any earlier link in the chain is carried on to the next and succeeding links, until it reaches the last, namely, man” (Heaton, 2001).

Advocates of organic agriculture maintain that the intensification of agriculture over the last 50 years has depleted the soil, and resulting crops, of nutrients, especially trace minerals, because the crops removed from the soil contain a wider variety of nutrients than farmers typically add back in form of chemical fertilizers (Heaton, 2001).

Data from the Department for the Environment, Food and Rural Affairs (DEFRA) shows that between 1940 and 1991, trace minerals in UK’s fruits and vegetables fell by up to 76 percent (McCance 1940-1991; Mayer, 1997) and United States Department of Agriculture (USDA) figures show similar declines (Bergner, 1997).

In organic agriculture, nutrients returned to the soil in manures and composts have to be cycled via the biological life of the soil before they become available to crops (Hodges, 1981). A gram of healthy soil can contain some 600 million microorganisms and tens of thousands of different species of bacteria and fungi (including mycorrhizae), as well as organic and inorganic matter, air, water and water vapour that undergo complex, far from fully understood, chemical reactions (Ingham, 2001). It is known that mycorrhizae proliferate in soil with a high organic matter content, such as that supplied in manure and composts, and that they form symbiotic associations with plant roots. Root uptake of nutrients is enhanced by mycorrhizae, due to the larger nutrient-absorbing surface provided by fungi. This area has been calculated to be up to ten times that of roots without mycorrhizal fungi (Tisdale, 1999).

In this way and through the actions of other microbiological soil life, plants are naturally supplied with a whole range of nutrients that would otherwise be too distant, insufficiently supplied or physically unavailable for uptake by plant roots alone. So in the organic farming the biological activity in the soil in fundamental to the breakdown of organic matter and the delivery of the range and quantity of nutrients required by the crop (Balfour, 1946).

The results of a 21 year field trial in Switzerland comparing organic and non-organic farming systems show dramatic differences in soil microbiology. The mass of microorganisms responsible for soil fertility and delivering nutrients to the roots of crops was up to 85 percent higher in the organically managed field than that non-organically managed (Fliessbach, 2000)

1.3. Food quality assessment

Consumer’s interest in healthy and save foods is continuously growing (Kahl, 2003). The development of the organic food market confirms that there is no doubt about it, that high quality foods are in increasing demand and the question of food quality has gained a high significance. Food traceability and organic food authenticity are important as consumers purchasing organic products should be able to do so with confidence. Nevertheless, at present there is no suitable and validated method for the authentication of organic foods and the distinction from the conventionally produced foods (Annual Report, 2005).

1.3.1. Standard approach to food quality

In the conventional vision, food quality mainly consists in the external, nutritive and sensory proprieties of the product (Burgt, 2005), as also in it’s safety (Heaton, 2002). The nutritive proprieties are strictly associated with the contents of different nutrients, minerals and vitamins, whereas the product’s safety is understood as the absence of undesirable and potential harmful substances.

Results of six main revives of comparison studies (Woese, 1997; Wortington, 1998; Diver, 2000; Brandt et al, 2000; Williams et al., 2000; Heaton, 2001) on the quality of organically and conventionally grown products show that in the majority of cases, the cultivation system influenced the product quality in terms of primary and secondary nutrient contents, nutritional value, product safety, and sensory proprieties in favor for the products deriving from the organically managed cultivation system (see chapter 2.5.). In many cases these differences can be pointed out by the use of the commune methods of analysis, that is by quantifying the singular substances, which are present in the product. Although results of many experimental studies showed that the standard view into food quality has to be extended (Strube et al., 2005).

1.3.2. Holistic approach to food quality

In the holistic approach to food quality the product is seen and understood as a whole. It was shown that singular substances do not provide the same impact on human health as whole comestibles. Antioxidant vitamin supplements for instance provide no cardiovascular benefit (Group, 2002; Vivekananthan et al., 2003) as well as supplementation with vitamins A, C and E and beta-carotene offers no protection against the development of lung cancer. Instead a diet rich in vegetables reduces the risk of lung cancer by approximately 25 percent. For the human health relevance have either the singular substances and their combined natural balanced occurrence and coherence. Past experience shows also that with the development of analytical and nutritional sciences more elements and aspects are found to be vital for health, through they may not even be measured at present (HDRA, 2001).

Consequently the quality of crops or products presents a very complex measure, which can be characterized on different levels: level of the singular substances and the whole, undivided product. Therefore the holistic approach to food quality can be regarded as a complementary viewpoint to the common approach; and both would be necessary to understand the food quality in a proper way.

1.3.3. The holistic quality concept

The holistic concept of food quality is not a new concept. It is more a reintegration of complementary theories of natural philosophy, which differ from the usual point of view, with the natural science. From the point of view of natural philosophy the holistic approach confronts the questions “what is life?” and “what characteristics does food supporting the life processes have to have?” (Rahmann et al., 2006). In the holistic concept foods are viewed as living substances, thus they are understood in a dual way, as integrities of matter and organizing or vital forces, which are beyond the matter, and which are the driving forces for all the life processes of the organism (Steiner, 1994; Balzer-Graf, 2000; Andersen, 2001).

In accordance to this concept of quality, the quality of comestibles is understood in a widespread way. In the Inner Quality Concept, investigated by the Louis Bolk Institute in Holland, life processes like growth and differentiation (including ripening) and their integration or balance are considered to have the primary importance in obtaining a healthy product (Bloksma, 2001).

Methods for examining the food quality seen in this sense of balance between the life processes are needed and they have an important meaning especially for the market of organic foods and products.

1.3.4. Holistic methods of food quality analysis

The maintenance of the samples structure is of crucial importance in the investigations on food quality by use of the holistic methods. The structural coherence or inner order of the analyzed food reflects its quality and the methods are calculated so as to depict the structure of a sample that makes evaluation possible. Therefore the down-process is minimized and the methods are regarding the product as a whole (Rahmann, 2006).

The picture forming methods provide a suitable tool to assess food quality holistically, which means that they do not concentrate on the content of singular substances present in food, but on the entire living organism, its constitution and organization. Dr. Ursula Balzer-Graf of the Swiss Institute of Vital Quality, who has worked with the picture forming methods for decades, explains: “Quality research in the field of living substances needs research methods appropriate to the phenomena of life. Not only the substance of foods but also the organizing activity therefore has to be included in proper scientific work. This is where these methods pick up as holistic quality research methods. The duality of substance and organizing activity are taken into account together and at the same time” (Balzer-Graf, 2000).

1.3.5. The picture developing methods

The picture forming methods consist of the biocrystallization method and two other chromatography methods (circular chromatography and ascending chromatography). As the name of these methods indicates, the output is not numerical data, but various types of morphological formations or “pictures”. In the case of the biocrystallization method the pictures are represented by ramified crystal structures arising on circular glass plates during the crystallization process. The chromatography methods outputs are colored, two-dimensional formations appearing on chromatography paper (Andersen, 2001).

These methods have been applied from the beginning of the picture forming methods, for over 70 years within the bio-dynamic and organic movements in different fields of research as the following :

- differentiation of farm produces and foods coming from different farming systems,

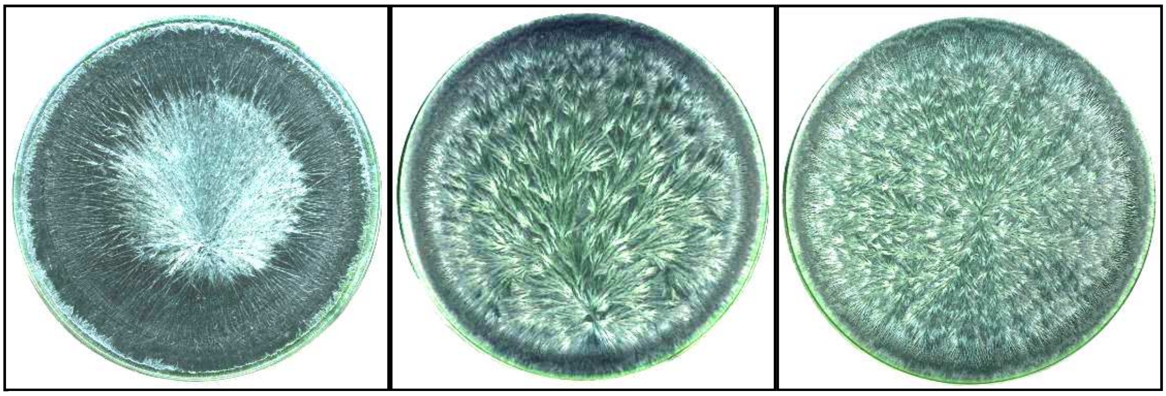
- comparison of different food processing and preservation techniques,
- specifications of soil qualities,
- specifications of compost maturity and quality,
- quality control of foods and farming produces,
- freshness test of foods and farming produces,
- comparisons of different crop varieties,
- differentiation between hybrid and seed fast varieties (Balzer-Graf, 2001, Balzer-Graf, 1995).
- differentiation of genetically modified crops.
- in anthroposophical medicine in diagnosis (blood crystallization) (Rozumek, 2004),

1.3.6. The biocrystallization method

The biocrystallization method – known also as the ‘cupper chloride crystallization’ and ‘sensitive crystallization’, is a holistic method and makes part of the so-called picture forming methods. Among the various picture forming methods biocrystallization has gained most recognition (Diver, 2000). Although at present the mechanisms of the method are not fully understood (Heaton, 2001), the huge number of investigations performed in the past and recently seems to prove the credibility of the method. The aspirations of German researchers for validating the biocrystallization method ended up successfully (Kahl et al., 2006b) and the support of computerized image analysis and tests deriving from the sensory analysis are permitting a standardized and reproducible evaluation of the crystal patterns (Andersen et al., 1999; Kretschmer, 2003).

The biocrystallization method was originally introduced by Ehrenfried Pfeiffer in 1931, and is based on the crystallographic phenomenon that when adding biological substances, such as plant extracts, to aqueous solutions of dihydrate copper chloride, biocrystallograms with reproducible dendritic crystal structures are formed during crystallization (Kleber, 1959). The crystal patterns obtained out of crystallization are specific for the admixed sample (Andersen *et al.*, 1998).

Whereas patterns resulting from crystallization of an aqueous copper chloride solution without additives shows a characteristic, unordered aggregation of $CuCl_2$ needles. When adding organic substances (plant juices or extracts) more or less coordinated forms of dendritically configured needle strands arise (Kretschmer, 2003). The resulting forms are dependent on the applied mixing ratio between the organic additive and the $CuCl_2$ solution. Determining of a suitable mixing ratio requires the application of concentration series, where patterns with fixed amount of reagent and an increasing amount of the admixed sample are generated, or a matrix procedure, where several concentration series are generated, with an increasing amount of each reagent (Andersen, 2003).



Picture 1: Biocrystallograms. A biocrystallogram of pure cupper chloride (picture on the left) shows a dense conglomeration of steams and needles, but not an organized structure. The biocrystallograms in the middle and on the right are prepared from durum wheat samples, respectively – grown organically and conventionally, coming from the MASCOT trial field experiment carried out at the Interdepartmental Centre for Agro-environmental Research "E. Avanzi" of the University of Pisa.

The crystallization process takes place in specifically designed chambers with controlled atmosphere (relative humidity and temperature). The inner chamber makes up the evaporation unit and the outer chamber ensures constant conditions.

One of the most important and difficult steps of this method is represented by the evaluation of the pictures in an objective way and then by linking that with a quality judgment. The evaluation of the biocrystallograms is traditionally carried out visually, by observing the patterns, or by the application of adequate computer software. The computer supported analysis leans on the grey level distribution of the scanned crystal pictures. The simple analysis of the grey level distribution delivers information about the degree by which the plate has been covered by structures during crystallization. Furthermore it can give results regarding both texture and structure of the analyzed patterns (Kretschmer, 2003). The visual evaluation is based on observation and description of the singular structure elements of the pictures and also of the overall character of whole patterns. Through decades of studies the visual evaluation has been approached by researchers in different ways (Selavry, 1957; Petterson, 1967; Enquist, 1970) and enabled a successful discrimination of the analyzed samples. In spite of that there still was no scientifically accredited method. At present often techniques deriving from the sensory analysis are applied and support the credibility of the visual evaluation. The most frequently applied technique is based on evaluation of the features in accordance to well defined criteria, like: integration, centre coordination, fullness with side needles, length of side needles, etc., as well as specifying their intensity in the analyzed crystal picture (Kretschmer, 2003).

That plant extracts exert some organizing force on the crystallization of metal salts seem to be clear (see picture 1), therefore many researchers claim that the biocrystallization method can visualize the organizing or architectural level of the sample (Heaton, 2001). Furthermore Andersen (2001) explains that the method delivers information about the whole organism, its physiological balance between anabolic and catabolic processes, about the formative forces behind the biochemical processes and about the degree to which the plant has realized its potential under the given conditions.

Making a review of the bibliography published on the biocrystallization subjects appears that the main fields of the method application are quality investigations and comparisons of crops grown under different conditions.

Mader et al. (1993) examined samples of beetroots grown under organic and non-organic (integrated) management in blind tests. The samples had to be grouped and identified according to their farming system using various picture forming methods, including biocrystallization. Though not perfectly, a high degree of differentiation and identification was possible in all three years investigated. In another experiment Alfoldi et al. (1996) tested blind samples of wheat and potatoes grown under organic and non-organic (integrated) conditions. A successful identification of all samples was reported. Granstedt and Kjellenberg (1997) compared the effects of non-organic and organic fertilizer treatments applied to a four year crop rotation in a 32 year field trial. The results of the biocrystallization method showed, that “the organization traits were consistently better in the organically grown samples than the non-organic ones”. In a milk study performed by the Louis Bolk Institute in Netherlands bulk tank milk samples were analyzed. The samples were gathered from five paired farms situated in the same regions: five organic and five conventional farms. The biocrystallization patterns were evaluated visually and by means of an image analysis software. The visual analysis illustrated a better integration and coordination, as also more perradiation and longer “side needles” in organic milk. The computer image analysis indicated a denser needle structure in organic milk in comparison to the conventional milk (Huber et al., 2005).

Besides the application of the biocrystallization method for distinction of samples deriving from different farming systems, the method found application in degradation tests, where it is used to monitor the decreasing quality of crop and food samples under stressful conditions. Andersen (2005) performed a study on biocrystallization of juices processed by microwave heating. In this experiment lead in collaboration between four biocrystallization laboratories: BRAD (Denmark), University of Kassel (Germany), Louis Bolk Institute (Nederland) and Kristallizationslabor (Switzerland) two organic carrot samples – a ripe and a sub-ripe sample – were exposed to a microwave heating procedure. The samples were successively heated up to 80-84 ºC and analyzed by the use of the biocrystallization method. The result showed, that the microwave heating caused a degradation of the picture developing properties, which are not observed for the untreated juice. The ripe sample had a significantly better ability to resist the loss of picture developing properties caused by the microwave heating than the sub-ripe one.

The method of biocrystallization, as also the other picture developing methods, has not gained any widespread scientific acknowledgement until now. The lack of a validated methodology and the need for more objective means of evaluating the pictures are mentioned in reviews on the numerous investigations conducted in the context of the biocrystallization method to be the main insufficiencies and difficulties on the way to a wider application of the method (Kretschmer, 2003).

On the other hand the biocrystallization method seems to be one of the most promising holistic methods for accessing the food quality being backed by a wealth of experiments performed in the history and several stages of optimization since the

1930’s. Recently a group of German researchers from the University of Kassel was able to validate the biocrystallization method (in-house validation), in accordance with the standards DIN ISO 17025, for the differentiation of crop samples deriving from different farming systems. In this study factors like repeatability, intermediate precision, reproducibility and robustness of the method were examined and specified (Busscher et al., 2004). In the study apple, carrot and wheat samples coming from organic and conventional farming systems were analyzed in a two year repetition. By means of the biocrystallization method it was possible to differentiate the samples and also to classify them (80-90 percent correct) in accordance to the different farming systems (Kahl et al., 2006a).

Furthermore the successful application of tests deriving from the sensory analysis supports the visual evaluation technique and increases its credibility. In a study about the establishment of a tool for controlled Visual Analyze in accordance with the ISO 658-1985 a trained panel was able to discriminate between crystal patterns obtained from fresh carrots and freeze dried carrots by the use of reference picture tests (Kretschmer , 2003).

Recently computer image analyze techniques are often used in the evaluation of received patterns. Suitable software was created by a group of Danish researches. In a project sponsored by the Danish Agricultural Ministry it was possible to correctly classify 21 patterns into seven groups representing each of seven days over which a carrot extract was allowed to degrade (Andersen et al., 1999).

Since the middle of the eighties the interest in the picture forming methods, as a tool for specifying the food quality, is continuously increasing (Tauscher et al., 2003). The chemical analysis, which reduces food quality solely to the determination of chemical compositions of foods often cannot show the interactions between food and human health and loose aspects, such as ‘vitality’. For this reason the holistic methods could represent an important support to the traditional analysis in giving a more global approach to the quality of foods. That has a particular meaning especially for the dynamically growing market of organic foods.

1.4. Wheat quality and human health

Wheat is grown in almost every temperate-zone country of North America, Europe, Asia, and South America. The largest wheat-producing counties are China, India, The United States of America, Russia, Canada, and Australia (Schnepf, 2008). Wheat’s quality has a great importance on human health because of the high intake of wheat products.

However the majority of the breeding practices performed on wheat varieties have as scope the creation of wheat varieties possibly adapted for common baking techniques or other commercial purposes. Therefore in most of the cases the high quality of wheat is understood in terms of high yielding and high protein content – especially high gluten content.

From the nutritional point of view the concentrations of albumins and globulins are important because of the essential amino acids contents of these grain protein fractions. The nutritional value of the so called storage proteins (gluten proteins) is low. Moreover the increasing consumption of gluten in wheat products, as also in other gluten containing products, and the early introduction of wheat in infant diet are resulting in a growing number of wheat intolerance and gluten allergy incidences in humans.

Recently there is a growing interest in the ancient wheat varieties and organically grown wheat.

1.4.1. Wheat protein composition

The protein quality of wheat grains is a parameter which is important for both: the nutritional value of wheat products and the technological quality of flour. From the nutritional point of view the content of albumins and globulins is crucial because of the greater occurrence of the essential amino acids in these proteins. Whereas the technological quality is mainly correlated with the contents of gliadins and glutenins, which have the ability to create the gluten complex during the preparation of dough.

The protein fractions of the wheat grain

The grain proteins differ in their physical and biological properties.

They can be divided in four protein fractions in accordance to their solubility, as comprehensive studies conducted 100 years ago by Osborn (1907) have shown:

- albumins are soluble in water;
- glubulins are soluble in salt solutions but insoluble in water;
- gliadins are soluble in 70-90 percent ethanol;
- glutenins are insoluble in neutral aqueous solutions, saline solutions or alcohol.

In terms of their biological function, the albumins and globulins are largely the cytoplasmic or metabolically active proteins and the gliadins and glutenins are largely storage proteins (Lasztity, 1984). Storage proteins occur mainly in endosperm and therefore are also the most numerous group of wheat flour proteins and the metabolically active proteins are found in the germ and pericarp-aleurone layers.

There are large differences in the amino acid composition of cytoplasmic and storage proteins. The storage proteins contain a large proportion of glutamic acid and proline and only a small proportion of lysine, arginine, threonine and tryptophan. Metabolically active proteins contain considerably less glutamic acid and proline and have higher proportions of lysine and arginine which give these proteins a higher nutritive value, but lower functional (bred making) properties (Spurway, 1988).

1.4.2. Response of different grain protein to N fertilization

Many studies showed that the N fertilization level is the most influencing factor regarding the wheat quality, although the degree of influence is governed by annual weather conditions and by residual soil N (López-Bellido et al, 2001). An other study conducted under Mediterranean conditions, where one durum wheat cultivar was grown over three years under varying N fertilizer levels (zero, 100, 150 and 200 kg ha-1) and in four different locations, showed that the N fertilization had the strongest effect on the protein concentration year by year. The location factor also influenced the protein concentration, but in a small degree (Garrido-Lestache et al., 2005). A positive effect of N fertilization level on protein concentration was reported also by Bianchi et al. (1987), where five durum wheat varieties were grown over two years under different N conditions (80, 120, 160, and 200 kg ha-1) and by Fowler (2003) who examined the grain protein concentration and grain yield in ten different common wheat cultivars grown under varying N fertilizer levels.

Souza et al. (2004) found in a study on the influence of wheat genotype, location and N fertilization level on quality traits (like total protein content), that the role of cultivar selection and location are most important in archiving a desired wheat quality, whereas the N fertilization level is much less important.

1.4.3. Food allergies and food intolerances – foodborne diseases.

Food allergies and food intolerances are diseases, whose prevalence is rapidly increasing among the population in the developed countries. WHO/FAO (2002) has estimated the prevalence of food allergies and food intolerances in Europe to be around 3 percent in adults and 8 percent in children.

Foods which cause the most severe reactions and most cases of food allergies are: cereals, crustacean, eggs, fish, peanuts, soybeans, milk, and tree nuts (INFOSAN, 2006).

Several factors can be considered among the causes responsible for the ever-increasing rate of food allergies and adverse reactions to food:

- The increasing exposure to pollution (Koren, O’Neill, 1998).
- The increasing exposure to residues of artificial substances in foods (Koren, O’Neill, 1998).
- The early exposure of newborn babies to a grater range of allergens and the possible sensitization of the fetus during pregnancy.
- The possible changes in the allergenicity of foods during industrial transformation.
- The larger use of food additives in processed food products (WHO/FAO, 2002).

1.4.4. Organic produced food and its possible value in the prevention of foodborne diseases.

Although there are very few studies available about the allergenicity of organic products compared to conventional products, there are many facts that indicate, that organic products could have a lower risk in triggering food allergies and food intolerances:

- Under Soil Association standards only four chemicals are allowed in sprays on organic crops, while in the conventional agriculture the use of 430 chemicals is allowed. Organic products contain therefore fewer pesticide residues (Baker et al., 2002).

- The use of food additives in the organic food production is restricted to only 32 of the 290 food additives approved for use across the EU. A wide range and large quantity of potentially allergenic or harmful additives can be avoided while eating organically (Balch et al., 1997).
- Organic foods are free from antibiotic residues, because the prophylactic and regular use of antibiotics is not permitted in organic standards of animal husbandry (WHO, 1997).
- The use of manure instead of chemical fertilizers results with a different protein composition of crops. Regarding wheat, the grain contain less gluten. In baking such technical disadvantage can be overcome, but at the price of using appropriate methods of brad making (e.g. using a sourdough starter instead of yeast) (FiBL, 2006).
- Crop varieties cultivated in organic agriculture are often more natural. The tendency in the conventional agriculture to develop new varieties with better technological and nutritional qualities through breeding, is substituted in the organic agriculture with the concern with food quality through sustainability.
- The organic agriculture does not contribute to the pollution of soil, water and air (FiBL, 2006).

In consumers' minds organic products seems to be associated with a lower allergy risk: the big amount of available internet resources on food allergies and intolerances often promotes the consumption of organic products; organic shops sell also non-allergic foods; and many kinds of non-allergic foods are produced out of organically grown products.

Purchasing organic products in any case is part of a life style which reflects a particular ideology and value system (Meier-Ploeger, 2005).

Two studies showed the influence of anthroposophic lifestyle, which includes factors like restrictive use of antibiotics, antipyretics, and often a biodynamic diet, on the occurrence of allergies, sensitizations and other diseases in children. Floistrup et al (2005) has found in a study conducted in five European countries, that children from Steiner schools in comparison with children from neighbored schools had a statistically significant reduced risk for rhinoconjunctivitis, atopic eczema, and atopic sensitization. A similar study conducted by Alm et all. (1999) on children aged 5-13 years at two anthroposophic schools in Sweden, compared with children of the same age at two neighboring schools showed differences between these two groups of children in terms of history of atopic and infectious diseases. Skin-prick tests and blood tests showed that the children from Steiner schools had lower prevalence of atopy than controls.

The positive influence of an organic diet on health has been reported in varied studies. A recent study conducted in the US showed, that rural Midwestern men, in whose urine were found high amounts of the pesticide diazinon, herbicides alachlor and atrazine are far more likely to have abnormal sperm than men with lower levels (Heaton, 2003/04). An analogue study conducted in Denmark showed that the sperm density of members of an organic farming association (OFA) had significantly higher sperm counts when compared with the sperm density of three different occupational groups (Abel, 1994).

1.4.5. The celiac disease

A prevalent disease is the gluten-dependent etheropathy – the celiac disease, causing severe damage to the gut of sensitive individuals unless gluten is completely eliminated from their diet. The affliction is not IgE-mediated, and therefore the celiac disease is often not indicated as an allergy (Hamer, 2005).

The celiac disease is a intestinal disorder caused by T-cell responses to peptides derived from the gluten proteins present in wheat. The toxic peptides have been found in both the glutenin and the gliadin proteins in gluten. The only cure for celiac patients is a lifelong gluten-free diet.

The number of people suffering from the celiac disease is difficult to estimate because of the variable presentation of the disease, particularly since many patients can have little or no symptoms (AHRQ, 2004). Currently, estimates are that approximately 0.9 – 1.2 percent of the Western population has the affliction (Ciclitria, 2003).

The early exposure of young infants to the gluten and the big amount of this protein complex present in the human diet are certain reasons for the ever-increasing rate of celiac disease incidences. The breeding of cereal varieties, especially wheat, is promoting varieties with stronger gluten and high protein contents, because these features improve the variety's baking qualities (Johanson, 1995). In fact recent wheat varieties differ significantly in their gluten content and gluten strength from the ancient varieties.

In the organic agriculture wheat varieties are often cultivated with lower gluten content of lesser strength. Additionally the use of manure instead of chemical fertilizers in organic agriculture results in lower nitrate availability for crops, what in turn leads to a lower storage protein content in cereals (Pedersen, 2002).

Studies show, that the quantity of storage proteins in wheat (gliadins and glutenins) depends strongly on the level of N fertilisation. High N level dominates the quantities of α-gliadins, γ-gliadins, and LMW subunits, and in lesser degree the quantities of HMW subunits and ω-gliadins (Wieser et all., 1998).

The lower content of storage proteins in organically grown wheat might therefore result in a lower toxicity of this products.

An other interesting investigation topic is the possible difference in the toxicity of the gluten complex proteins among organic and conventional wheat varieties. Until now there are no available studies on the possible differences between organically and conventionally grown wheat in celiac disease triggering proprieties, and allergenicity influenced by the cropping system.

2. AIMS OF THE THESIS

The aim of the PhD thesis was to compare the qualities of *Triticum durum* and *Triticum aestivum* grown under conventional and organic conditions by means of three types of analysis methods: the common analysis, the sensorial analysis, and the holistic analysis. The scope of the introduction of these three groups of analysis was the completion of results coming from different assessments in order to receive a possibly wide view on the samples quality.
The different methods were concerned as complementary methods.

The scope of the **common analysis** was to quantify the contents of chosen substances in the analysed grain samples and gain results about the yields and technological parameter of dough. The results were compared depending on the specie, cultivation system and harvesting year (2005, 2006, and 2007). This part of the study was based on the common view on food quality and was carried out in frames of experiment I.

Experiment I: The aim of this experiment was the measurement and comparison of following parameters: yield, total protein content, gluten content, grain ash content, total phenolic content, and carotenoid content (lutein and zeaxanthin), weight of 1 hectoliter, and the Alveographic parameters of dough.

The **sensory analysis** of bread leafs baked out of conventional and organic flour was performed with the scope to gain results about the consumer preferences regarding sensorial attributes of organic and conventional bread. The sensory analyze, where the senses of humans are becoming the analyzing tool, was considered in this study as a kind of “bridge” between the common and holistic approach to food quality.

Experiment II: In this experiment organic and conventional bread was baked out of the *Triticum aestivum* samples (harvest 2005). The loafs were analyzed in a simple preference test by more then 150 consumers. The results regarding the consumers preferences and opinions were collected in questionnaires.

Among the **holistic methods** the biocrystallization analysis was chosen as a widest spread and studied method for quality analysis. It was performed with the scope to analyze the picto-morphological proprieties of the organic and conventional wheat grains and to gain information about their coherence and state. The picto-morphological proprieties of the samples give in the biocrystallization method issue to quality evaluation in terms of sample's structuring and ordering forces. This part of the study was based on the holistic vision on food quality.

Experiment III: The grain samples were analyzed by means of the biocrystallization analysis (harvesting years 2005, 2006). The aim of the experiment was the complementation of data collected in previous experiments by the information about the quality of the grain samples viewed as a whole.

Experiment IV: The gluten isolated mechanically from the organically and conventionally grown *Triticum aestivum* samples (harvest 2005) was analysed for his picto-morphological proprieties by means of the biocrystallization method. The quantity and strength of gluten in known to be influenced strongly by the cultivation system. This analysis had as scope to show if there are any differences in the structural phenomena of gluten isolated from organic and conventional grains.

Experiment V: This experiment consisted in the analysis of fresh and degradated grain extracts of the both species in question (harvest 2005) in dependence on the cultivation system. The scope of this degradation test was to analyze the samples prevention capacities in the ageing process.

3. EXPERIMENTAL PART

3.1. Forewords to the experimental part

3.1.1. The CIRAA Centre and Foreign Institutions

The research was conducted at the Interdepartmental Centre for Agro-environmental Research (*Centro Interdipartimentale di Ricerche Agro-Ambientali*) "E. Avanzi" of the University of Pisa in the period from January 2005 to December 2007.

A part of the here presented research was conducted at foreign institutions. The analysis of the secondary nutrients (lutein and zeaxanthin) was performed at the University of Kassel / Germany at the Department of Organic Food Quality and Food Culture in Witzenhausen and the biocrystallization analysis of gluten – at the Biodynamic Research Association Denmark (BRAD) in Herskind / Denmark.

3.1.2. The MASCOT (Mediterranean Arable Systems COmparison Trial) long-term experiment

The analyzed grain was cultivated organically and conventionally in a three year repetition in 2005, 2006 and 2007 as part of the MASCOT long-term trial.

The MASCOT (Mediterranean Arable System COmparison Trial) long-term experiment was planned in the late 1990s with the aim to establish the first exhaustive and scientifically rigorous Mediterranean long-term experiment comparing organic and conventional management systems for a typical arable crop rotation of Tuscany coastal plain (Mazzoncini, 2002a and 2002b).

The experiment is based on a stockless system. Although the stockless management is not an ideal model for the organic agriculture, it reflects the real farm situations of many mediterranean areas. The MASCOT experiment meets therefore the need of farmers and contributes to the scientific knowledge on how to best manage stockless organic systems.

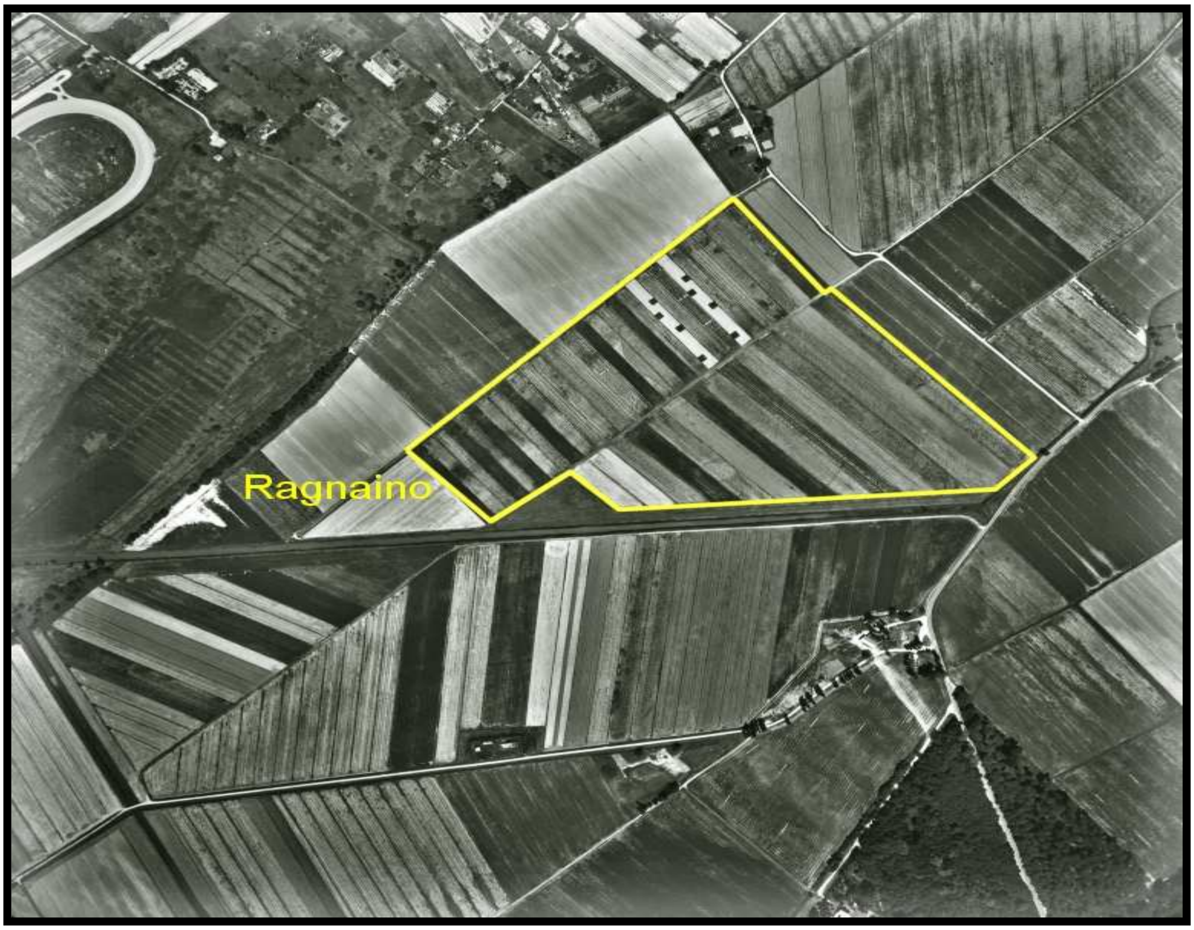
The experiment was started as a long term research in 2001 and is carried out at the Interdepartmental Centre for Agro-environmental Research "*E. Avanzi*" (CIRAA) of the University of Pisa. CIRAA, with its 1700 ha area, is the largest experimental centre in Italy, and one of the largest in Europe.

CIRAA is located on the northern cost of Tuscany, 8 km SW of Pisa and 1 km E of the Tyrrhenian Sea on the southern side of the Arno river (lat. 42º41', long 10º23'), and is included within the borders of the Migliarino-San Rossore-Massaciuccoli Natural Park.

CIRAA lies on plain (1 m a.s.l.), deep and stone-free soils that have originated from particle depositions occurred through periodic overflows of the Arno river and land reclamation carried out during the Medici age (15th century). The soils are, consequently, young and heterogeneous, ranging from sandy (> 82% sand) to clayey (40-44% clay), with a high presence of silty-loam soils. The MASCOT experiment is situated on loamy soils. The climate is typical for the Mediterranean area - the mean values for the monthly air temperature are 11℃ in February and 30℃ in August. The rainfall is about 853 mm/year and is concentrated mainly in autumn (October-November) and spring (April-May).

The MASCOT experiment is arranged on an area of ca. 24 ha, which is divided into fields of 0.35-1 ha each, where five crops (sugar beet, sunflower, pigeon bean, common and durum wheat) are allocated and managed organically or conventionally. Each group of five fields represents a system within a block and each crop is present every year. Systems are replicated three times according to the RBC design.

The aim of the MASCOT experiment is to evaluate the long term dynamics of soil parameters, such as physical, biological and chemical parameters of macronutrients (NPK), of weed and pest populations and communities, of system biodiversity as well as economic and energetic aspects of systems management and effects on produce quality.



Picture 2: The MASCOT Experiment

	Crop									
	Sugar beet		Common wheat (+ red clover in OS)		Sunflower		Pigeon bean		Durum wheat (+ red clover in OS)	
Cultural practice	CS	OS	CS	OS	CS	OS	CS	OS	CS	OS
Main tillage (depth)	Ploughing (50 cm)	Ploughing (25 cm)	Ploughin (25 cm)	Ploughing (25 cm)	Ploughing (25 cm)	Ploughing (25 cm)	Ploughing (25 cm)	Ploughing (25 cm)	Ploughing (50 cm)	Ploughing (25 cm)
Fertilisation	Mineral	Organic	Mineral	Organic	Mineral	Organic	Mineral	None	Mineral	Organic
Fertilizer rate	162 N 138 P_2O_5 250 K_2O	30 N* 30 P_2O_5* 30 K_2O*	156 N 92 P_2O_5 30 K_2O	30 N* 30 P_2O_5* 30 K_2O*	128 N 96 P_2O_5 96 K_2O	30 N* 30 P_2O_5* 30 K_2O*	0 N 96 P_2O_5 0 K_2O	-	156 N 92 P_2O_5 0 K_2O	30 N* 30 P_2O_5* 30 K_2O*
Seeding rate	18 seeds m^{-2}	18 seeds m^{-2}	200 Kg ha^{-1}	200 Kg ha^{-1}	8 seeds m^{-2}	8 seeds m^{-2}	200 Kg ha^{-1}	200 Kg ha^{-1}	230 Kg ha^{-1}	230 Kg ha^{-1}
Weed control	Pre-e + Post-em	Pre-e (banded)+ Post-em	Post-em	Spring-tine harrowing	Pre-em	Hoeing	None	Spring-tine harrowing	Post-em	Spring-tine harrowing
Pest control	Curative	Curative	None	None	Curative	None	None	None	None	None
Irrigation	None	None	None	None	None	None	None	None	None	None
Crop genotype	Puma[1]	Puma[1]	Bolero[2]	Bolero[2]	Carlos[3]	Carlos[3]	Vesuvio	Vesuvio	Claudio	Claudio
Residue management	Incorp.	Incorp.	Removed.	Incorp.	Incorp.	Incorp.	Incorp.	Incorp.	Removed.	Incorp.

Table 2: Cultural practices applied in the conventional (CS) and organic (OS) management systems.
[1] Early cultivar, [2] Bread cultivar, [3] High oleic acid cultivar. Pre-em, post-em = Pre- and post-emergence herbicide application, respectively, Incorp. = incorporated; * from dried manure; the amount of nitrogen available in OS after clover was estimated 70 kg ha^{-1}.

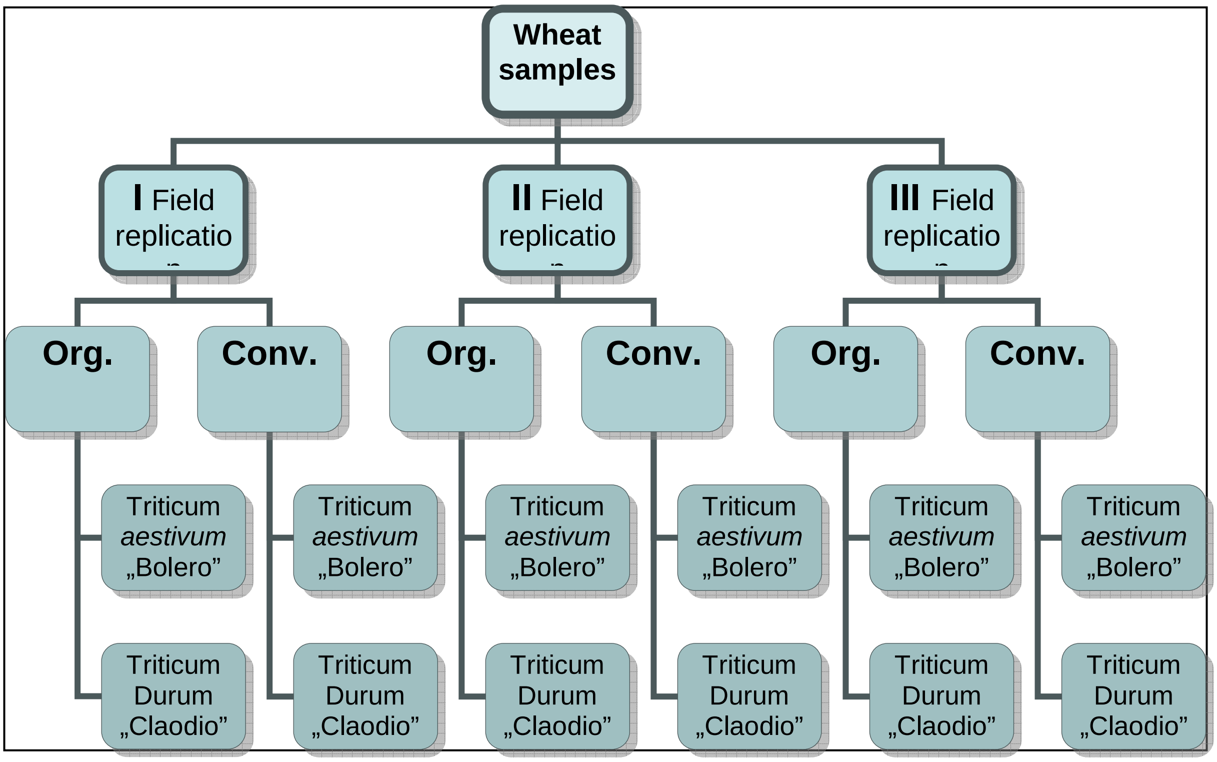
3.1.3. Specification of the examined wheat samples

The wheat samples derived from the MASCOT trial. The examined samples were of two different wheat species:

- the common wheat (*Triticum aestivum*) variety Bolero
- the durum wheat (*Triticum durum) variety* Claudio,

and there were twelve wheat samples in total per year: six of the variety Claudio, and six of the variety Bolero.

The samples collected from each variety derived from the organically cultivated system (three field replicates) and from the conventional cultivation system (three field replicates) (see graphic 4).



Graphic 4: Description of the examined wheat samples, deriving from the MASCOT trial.

In the MASCOT experiment the N in the organic system was assured by the use of pigeon bean in the crop rotation (before durum wheat) and the inter-sowing of red clover in common wheat. Additionally 30 kg ha^{-1} N, 30 kg ha^{-1} P_2O_5 and 30 kg ha^{-1} K_2O fertilizer from dried manure was applied. The amount of nitrogen available in the organic system after clover was estimated at 70 kg ha^{-1}. The fertilization in the conventional system was on a mineral basis with the following amounts of fertilizer: 156 kg ha^{-1} N and 92 P_2O_5 kg ha^{-1} in both wheat's, in addition to 30 and 0 kg ha^{-1} K_2O in common and durum wheat's respectively.

3.2. Experiment I – Wheat grain yield and grain quality as determined by conventional methods of analysis

3.2.1. Wheat grain yield

Background

It is well known that the yield and protein content of wheat depends in a significant degree on the N fertilization. Therefore these two parameters tend to differ in dependence on the cropping system and on the applied crop management.

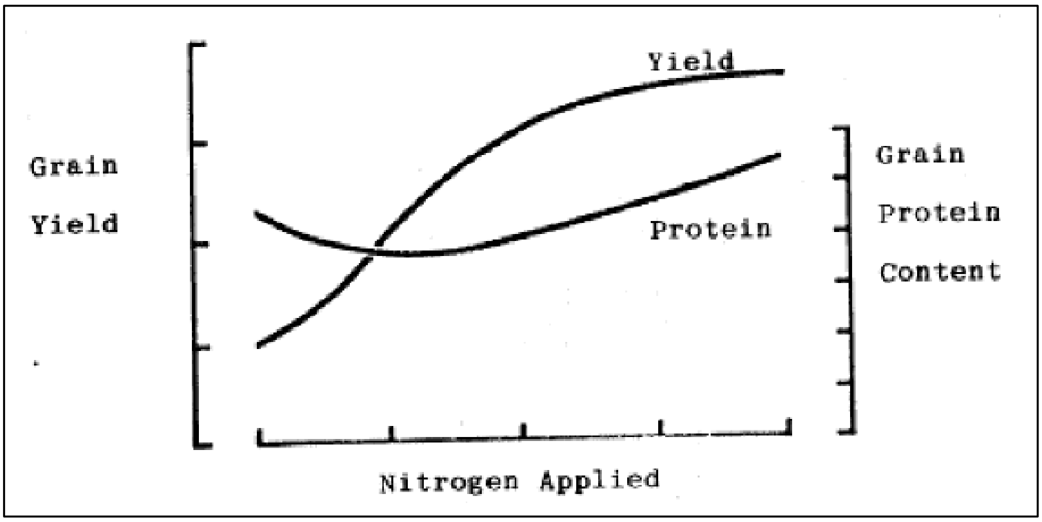
These two parameters are often associated with the term of wheat quality, that is the higher the yield and protein content the better the quality. From the nutritional point of view quality is not correlated with the total protein content and yield, but rather with the quality of proteins. Moreover it is rather the opposite to the technological quality.

It currently becomes more important for conventional growers to be able to produce quality wheat for specific markets, which have well defined technological quality requirements.

This challenge requires management of N fertilizer for both grain yield and grain protein content. Studies confirm that the response of yield and protein to N fertilization is similar for all classes of wheat (Subedi, 2007) and can be described as follows:

Yield often doubles with modest rates of N fertilizer when N is deficient and yield is not limited by water supply. Grain protein content usually decreases with the initial increase in grain yield because protein is “diluted” in a larger quantity of grain. Consequently, grain protein percentage is often higher in unfertilised controls than in modestly fertilized plots. At higher rates of N, grain yield response begins to taper off and grain protein content increases. Grain protein continues to increase as N supply exceeds the rate required for maximum yield.

The relationship between yield and protein responses is such that there is a range of grain protein concentrations associated with near maximum grain yield. This allows to manage N fertilizer for protein concentrations acceptable in different classes of wheat.



Graphic 5 response of grain yield and grain total protein content to different levels of N fertilization (Thomison, 2004).

Differences in grain yield due to varying N fertilizer rates were reported in numerous studies. Sternam (1990) found in his three years study about the influence of N fertilizer on yield and protein content in grains, that the lowest yields in all cultivars were obtained, where no nitrogen was applied, whereas the nitrogen inputs of 140-180 kg ha^{-1} resulted in highest yields.

Indeed the lower yields in the organic system are often reported as one of the disadvantages of this cultivation method. Leake (1999 a) reported that organic wheat, beans and peas yields were 60-70% whereas oats were 85% of conventional yields. Results of the DOK experiment in Switzerland – a 21-year study – show, that the organic yields were on average only 20% lower then the conventional ones, whereas the input of fertilizer and energy in the organic system was reduced by 34 to 53% with a 97% drop of pesticide input. Enhanced soil fertility and higher biodiversity found in organic plots indicated that the organic system was less dependent on external inputs (Mäder, 2002). Although organic farms achieve lower yields, when compared with high intensity conventional farms, some of these losses can be offset against savings on expensive fertilizers and insecticides (Podger, 1999).

Materials and methods

The yield data for the common and durum wheat deriving from the MASCOT long-term experiment are collected since the beginning (year 2002). The common wheat has been introduced into the rotation one year after the onset of the long-term trial.

The statistic analysis of the data was an ANOVA analysis with a randomized block design. The means of the treatments were compared by use of the LSD test. For the analysis of the effect of the variable “year” on the different characteristics a split-plot design was used, were “year” was considered as a secondary factor.

Results

The wheat productivity in years 2005-2007 is shown in the tables below. In average, considering all 3-year periods, the organically managed system determined a decrease in yield of 27% and 37% in durum and common wheat respectively. The grain yield of the durum wheat was lower by 18% in 2005, by 23% in 2006 and by 57% in 2007 whereas the yield of the common wheat was lower by 54% in 2005, by 19% in 2006 and by 41% in 2007.

The yields were affected by the climatic conditions of the different cropping seasons, in fact they were generally higher in 2005 and 2006, when a good water supply was guaranteed by regular rainfall all along the growth cycle, while the temperatures did not diverge from the typical ones for the area. In 2007 the abundant precipitations during the sowing time and the flowering phase created some difficulties to the development and yield construction of the crop. Also higher temperatures (1-2 °C of respect to usual) were influential.

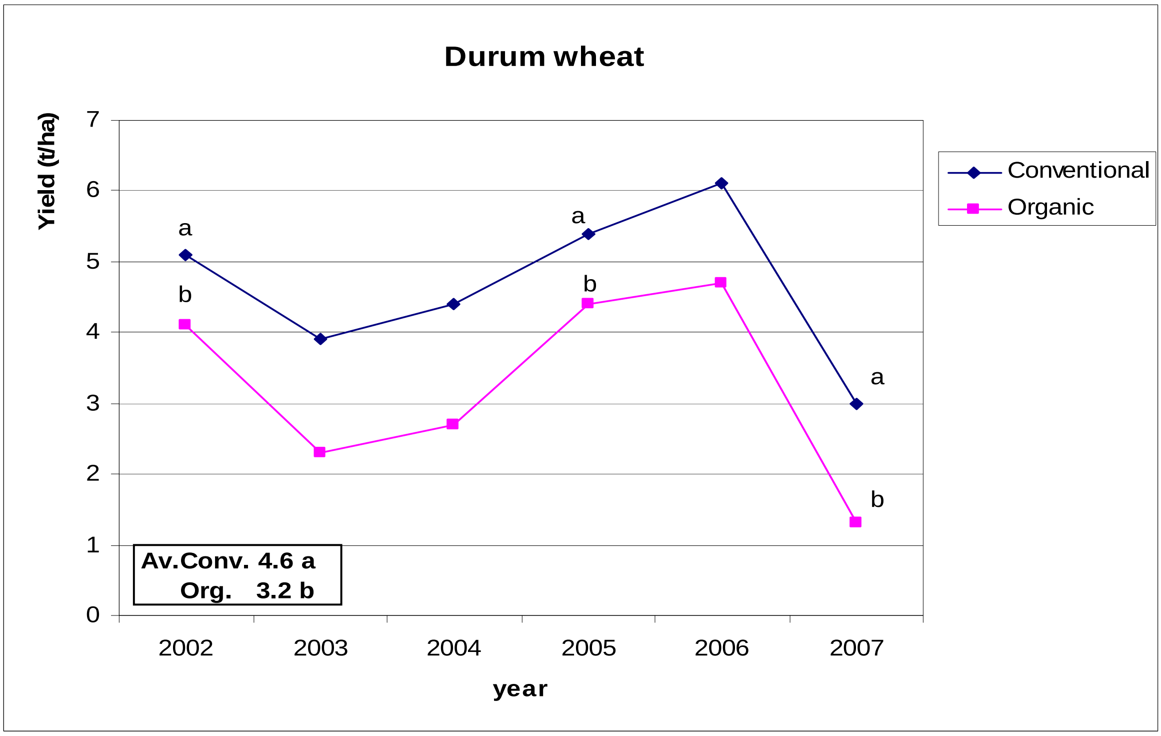
	***Triticum durum,* Yield t/ha**			
	2005	2006	2007	mean
conventional	5.4	6.1	3.0	4.8
organic	4.4	4.7	1.3	3.5
significance	*	ns	**	*
mean	4.9	5.4	2.1	*

Table 3: Durum wheat grain yields in 2005-2007.

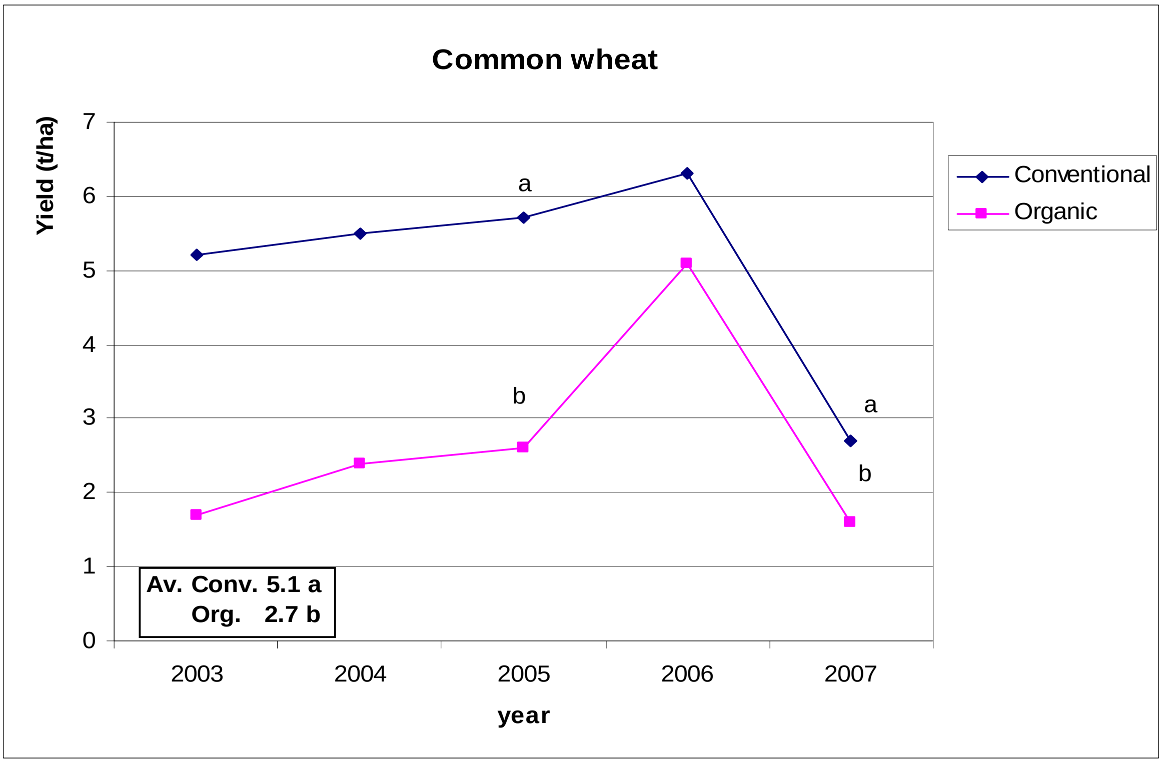
	Triticum aestivum, **Yield t/ha**			
	2005	2006	2007	mean
conventional	5.7	6.3	2.7	4.9
organic	2.6	5.1	1.6	3.1
significance	**	ns	*	**
mean	4.1	5.7	2.2	**

Table 4: Common wheat grain yields in 2005-2007.

The grain yields from the beginning of the MASCOT long-term trial are shown in the graphs below. The durum wheat yields decreased in 2003, i.e. in the second year after the conversion period and showed a continuous increase in the successive years. The organic durum wheat yields were always lower than the conventional ones by ca. 1-2 t/ha. The common wheat however was introduced into the crop rotation in 2003 and as the durum wheat showed in this year the lowest yield and a continuous increase during the successive years (until 2006). Differences between the two systems were more appreciated in common wheat where the organic systems determined a decrease in yield of about 47% in average. For this specie the differences between the two systems seems to be more emphasized in the years 2004 and 2005 where the highest temperature in the flowering and ripening phase seems to have had a more significant repercussion on the organic wheat. On the contrary durum wheat seems to be more suitable to the organic systems (the yield reduction in the organic variant was lower than in common wheat and amounted to 30%).



Graphic 6: Organic and conventional Durum wheat yields 2002-2007.



Graphic 7: Organic and conventional common wheat yields 2002-2007.

Conclusions

The two cropping systems affect the yield of wheat as was expected, in particular the organic system determined a decrease in yield of about 30%. In particular durum wheat seems to be the most suitable to the nutritional condition created from the organic system. Since the differences between the two systems are always present a trend can be identified in both systems, a continuous increase after the conversion period towards sustainable level of yield.

3.2.2. Wheat grain protein content

Background

The grain protein content is an important technological quality indicator of wheat. Especially the amount of glutenins and gliadins is of greatest interest, because of the ability of these proteins to create the gluten complex. Many studies conducted on wheat grown under different N conditions confirm the relation between N rate and protein content. López-Bellido (2001) found a positive correlation between the N fertilization rate and the protein content in grains, although the degree of influence is governed by annual weather conditions and by residual soil N. Another study conducted under Mediterranean conditions, where one durum wheat cultivar was grown over three years under varying N fertilizer levels (0, 100, 150 and 200 kg ha^{-1}) and in four different locations, showed that the N fertilization, followed by year, had the strongest effect on the protein concentration. The location factor also influenced the protein concentration, but in a small degree (Garrido-Lestache et al., 2005). A positive effect of the N fertilization level on protein concentration was also reported by Bianchi et al. (1987), where five durum wheat varieties were grown over two years under different N conditions (80, 120, 160, and 200 kg ha^{-1}) as well as by Fowler (2003) who examined the grain protein concentration and yield in ten different common wheat cultivars grown under varying N fertilizer levels.

Subedi et al. (2007) found in his study about the response of yield and grain protein content to different levels of N fertilization conducted on two different locations with different soil characteristics, that: sandy loam soil and clay loam soil, that the grain protein content depended mainly on the soil type and in a smaller degree on the N fertilization level. The grain protein content was significantly higher in both years in crops grown on clay loam soils, than on sandy loam soils.

Materials and methods

The samples were examined for the N content of grain by the use of the Kjeldahl method. The grain protein content [N x 5,75] was calculated for each field replicate and cultivation system.

The statistic analysis of the data was an ANOVA analysis with a randomized block design. The means of the treatments were compared by use of the LSD test. For the analysis of the effect of the variable “year” on the different characteristics a split-plot design was used, were “year” was considered as a secondary factor.

Results

As was expected the two wheat species in all the examined years differed significantly in their protein contents depending on the cropping system (see tables below).

The protein content of the organically grown grains was 18-20% lower then in conventionally grown grains. The two species showed approximately the same protein content (11 and 12% in average for durum wheat and common wheat) and in both species the protein content increased, also in the organic variants, from 2005 to 2007. It is interesting to note that the protein content was generally higher in these years, in which the lowest yields were noticed. Moreover the different nitrogen management of the organic system seemed to determine a more significant increase in protein content than the conventional one.

	Protein content (%), *Triticum durum*			
	2005	2006	2007	mean
conventional	10.9	12.5	12.1	11.8
organic	8.1	8.9	12.2	9.7
significance	*	ns	ns	**
mean	9.5	10.7	12.1	*

Table 5: Total protein content (%) in durum wheat.

	Protein content (%), *Triticum aestivum*			
	2005	2006	2007	mean
conventional	11.8	12.3	14.5	12.9
organic	8.5	10.1	12.0	10.2
significance	ns	*	**	*
mean	10.2	11.2	13.3	*

Table 6: Total protein content (%) in common wheat.

Conclusions

It has been found, that especially in stockless organically managed cultivation systems N is the main yield limiting factor. The protein content of wheat grown for the three years analysed seems not to fully confirm these findings. In fact there is a positive trend in grain protein content in both systems, despite a very different N supply – this aspect needs to be attentively deepened by further studies. In the study of Ruhe et al. (2003) the N delivery depends in a high degree on the percentage of legumes in the applied crop rotation, what explains the significant increase in protein content of the organic system where the leguminous crops are provided not only in crop rotation but also as green manure.

3.2.3. Technological parameter of grain

Background

Baking quality of wheat in commercial cultivars generally depends on the protein concentration of the grain (Gate, 1995; Goodings et al., 1997). Mediterranean areas are suitable for the production of high quality bread-making wheat (*Triticum aestivum* L.) because of the high temperatures during grain filling. Wheat quality is also influenced by variety and can be enhanced through the use of N fertilizer (Lloveras et al., 2001).

The technological quality of wheat from organic farming differs in many aspects from the one of conventional farming. The most significant differences are to be found in the content of crude protein and in parameters that characterize the wheat’s protein complex quality (Krejcirova et al., 2007). Higher nitrogen levels require a later vegetation phase, when the grain is forming and maturing. In organic farming with absence of fast-effect industrial fertilizers, a nitrogen deficit and a lower accumulation of wheat storage proteins are frequently experienced (Prugar, 1999). Wheat storage (gluten) proteins create up to 80% of the total amount of wheat grain (Shewry et al., 2000). Technologically, glutenins and gliadins are the most important wheat storage proteins. Their proportion and amount in wheat grain is variable and changes with the total protein content. The gluten quality for the specific final utilization is determined especially by the optimal combination of storage proteins. Each of them affects rheology in a unique way – dough viscosity and tensibility are affected by gliadins and elasticity by glutenins (Bushuk et al., 2002).

The quantity of the different protein fractions in grain, which influence the grain baking quality, differs depending on the N fertilization levels. A study conducted by Abrol et al., (1971) showed that the enhanced protein content observed, following increased fertilizer application, is mainly accounted for by an increase in gluten content. Bran and shorts have a higher percentage of protein than the flour. However, accumulation at high fertility takes only place in the flour. Protein fractionation studies reveal that there is an increase in the relative proportion of prolamin and glutelin while cytoplasmic proteins show little change.

Souza et al. (2004) in a study on the influence of wheat genotype, location and N fertilization level on quality traits, like total protein content, found however that the role of cultivar selection and location are most important in achieving a desired wheat quality, whereas the N fertilization level is much less important.

The differences of protein concentration in dependence of the selected cultivar were investigated in a study conducted by Maque et al (2004). It was showed, that the different cultivars of durum wheat varied in their protein concentration by the same nitrogen fertilizer level and same growing conditions from 130 to 164 g kg^{-1} at one location and from 146 to 186 g kg^{-1} at the second location.

Nevertheless other studies showed that the N fertilization level is the most influencing factor regarding wheat quality, although the degree of influence is governed by annual weather conditions and by residual soil N (López-Bellido et al., 2001). An other study conducted under Mediterranean conditions, where one durum wheat cultivar was grown over three years under varying N fertilizer levels (0, 100, 150 and 200 kg ha^{-1}) and in four different locations, showed that the N fertilization level, followed by year, had the strongest effect on the protein concentration. The location factor also influenced the protein concentration, but in a small degree (Garrido-Lestache et al., 2005). A positive effect of N fertilization level on protein concentration was reported also by Bianchi et al. (1987), where five durum wheat varieties were grown over two years under different N conditions (80, 120, 160, and 200 kg ha^{-1}) and by Fowler (2003) who examined the grain protein concentration and grain yield in ten different common wheat cultivars grown under varying N fertilizer levels.

Materials and methods

The analysis of the main technological and chemical properties (W, P/L, L, gluten content, weight of 1 hectolitre of grain, gluten index, SDS, ashes content) of the organic and conventional *Triticum durum* and *Triticum aestivum* wheat samples were carried out at the laboratories of ISC (*Istituto per la Cerealicoltura*) at Sant'Angelo Lodigiano (LO) in Rome.

The technological proprieties of dough, like strength (W), extensibility (L), and resistance to stretching (P), were measured in an Chopin Alveograph. Gluten content and gluten index were measured in accordance with UNI 10275 by use of the Glutomatic apparatus. The weight of 1 hectolitre of grains was weighted on a Schopper balance in accordance with UNI 10281, and the ashes content was measured in accordance with UNI ISO 2171. SDS represent the volume of the sediment of a suspension of flour in a lactic acid and sodium dodecilsulphate solution.

The statistic analysis of the data was an ANOVA analysis with a randomized block design. The means of the treatments were compared by use of the LSD test. For the analysis of the effect of the variable "year" on the different characteristics a split-plot design was used, were "year" was considered as a secondary factor.

Results

Values of weight of 1 hectolitre don't seem to be particularly affected by the cropping system in both species. In all the examined years and in both systems they reached the standard values reported by the data bank of INRAN (the National Nutrition Institute) for the corresponding varieties.

	Weight of 1 hl (kg), *Triticum durum*			
	2005	2006	2007	mean
conventional	80.7	83.0	82.7	82.1
organic	77.4	82.6	83.1	81.0
significance	ns	ns	ns	Ns
mean	79.1	82.8	82.9	*

Table 7: Effect of cropping system on 1 hl weight in durum wheat

	Weight of 1 hl (kg), *Triticum aestivum*			
	2005	2006	2007	mean
conventional	82.8	81.3	82.1	82.1
organic	80.7	80.2	80.0	80.3
significance	ns	ns	ns	Ns
mean	81.8	80.8	81.1	*

Table 8: Effect of cropping system on 1 hl weight in common wheat

The SDS (Sodium Dodecilsulphate Sedimentation) gives a measure of the gluten strength and seems to be positively related to the protein content. According to our experimental findings it showed to be lower in organic system than in conventional ones for both species. Values for both systems are however included within the range reported for the cultivars utilized. Gluten content and gluten index in durum wheat revealed in both systems values lower and higher respectively than those reported for the variety Claudio. Organic system highlighted gluten content values slightly lower than the conventional one, while for gluten index the effect of cropping system is yet to be clarified.

	SDS (ml), *Triticum durum*			
	2005	2006	2007	mean
conventional	55.7	45.0	24.0	41.6
organic	28.3	35.0	28.0	30.4
significance	*	*	*	*
mean	42	40	26	**

Table 9: Effect of cropping system on SDS sedimentation test in durum wheat

	SDS (ml), *Triticum aestivum*			
	2005	2006	2007	mean
conventional	51.0	60.0	56.7	55.9
organic	30.0	46.3	48.0	41.4
significance	ns	ns	ns	*
mean	40.5	53.2	52.4	**

Table 10: Effect of cropping system on SDS sedimentation test in common wheat

	Gluten content (%), *Triticum durum*			
	2005	2006	2007	mean
conventional	9.7	8.4	8.7	8.9
organic	5.9	7.6	7.6	7.0
significance	ns	**	ns	*
mean	7.8	8.0	8.2	**

Table 11: Effect of cropping system on gluten content in durum wheat

	Gluten index, *Triticum durum*			
	2005	2006	2007	mean
conventional	83.0	94.7	85.0	87.6
organic	83.7	95.7	92.0	90.5
significance	ns	ns	ns	ns
mean	83.4	95.2	88.5	ns

Table 12: Effect of cropping system on gluten index in durum wheat

No clear connection appears between the cropping system and the gluten strength measured by W in durum wheat, while the organic system always showed lower values in aestivum.

The ratio between resistance to stretching and extensibility P/L showed in general higher values in the organic system.

	W (J 10^{-4}), *Triticum durum*			
	2005	2006	2007	mean
conventional	98.3	130.0	80.0	103.0
organic	110.0	100.0	140.0	117.0
significance	ns	ns	*	ns
mean	104.2	115.0	110.0	*

Table 13: Effect of cropping system on W values in durum wheat

	W (J 10^{-4}), *Triticum aestivum*			
	2005	2006	2007	mean
conventional	145.0	229.0	270.0	215.0
organic	66.0	158.0	199.0	141.0
significance	ns	ns	ns	**
mean	106.0	193.0	234.0	**

Table 14: Effect of cropping system on W values in common wheat

	P/L, *Triticum durum*			
	2005	2006	2007	mean
conventional	0.4	1.1	1.3	0.9
organic	0.7	2.2	1.2	1.4
significance	ns	ns	ns	ns
mean	0.6	1.7	1.3	**

Table 15: Effect of cropping system on P/L values in durum wheat

	P/L, *Triticum aestivum*			
	2005	2006	2007	mean
conventional	0.5	0.6	1.0	0.7
organic	0.9	1.3	1.7	1.3
significance	ns	ns	ns	*
mean	0.7	1.0	1.4	**

Table 16: Effect of cropping system on P/L values in common wheat

The ashes content in durum wheat revealed very similar values. Even if significantly different in both systems, still in line with the expected values for the cultivar Claudio. In a three years average the conventional system seems to produce a slightly higher content of ashes.

	Ashes content (%), *Triticum durum*			
	2005	2006	2007	mean
conventional	1.8	1.8	2.1	1.9
organic	1.8	1.7	2.0	1.8
significance	ns	ns	*	*
mean	1.8	1.8	2.0	**

Table 17: Effect of cropping system on ashes contents in durum wheat

Conclusions

Since there is a strong association between the protein content and the bread production qualities, the organic system, in general, showed gluten strength values lower than the conventional one (see SDS, W), especially in common wheat. This will have a reflex mainly on the bread volume. Then the P/L values indicate that the obtained bread will probably have a harder consistence. Gluten quality, as indicated by the gluten index, measured in durum wheat, seems not to be so affected by the cropping system.

The solution to lower protein and gluten content which leads to lower elasticity of organic dough is achievable by adjusting the baking process in order to meet new requirements. In organic bread production more flour per loaf and longer rising times are needed in comparison to conventional production (Brandt, 2005). Also the milling process can influence bread quality. Kihlberg et al (2004) showed that the milling technique (stone-mill versus roller-mill) had a great influence on baking performance, followed by farming system (organic versus conventional), flour type and mixing intensity (low versus high). Therefore despite the worse bread-making quality of wheat from organic growing, it is not possible to say that it is unsuitable for bread-making

3.2.4. Secondary plant metabolites

Background

The knowledge about the secondary pant metabolites has not more than 30 years. The view on the components of food, which are water, carbohydrates, proteins, fats, minerals, vitamins, dietary fibers and non-nutrient substances, is still the same, but the understanding and appraisal of dietary fibers and non-nutrient substances, which means substances without a nutritive function, has changed totally (Watzl, 1995).

The non-nutrient substances, known recently as secondary plant metabolites or phytochemicals are a large, chemically diverse and still not fully discovered group of ca. 5.000 - 10.000 substances (Heaton, 2001). In contrast to primary plant products the phytochemicals are synthesized during the secondary metabolism, are present in plant tissues only in very little quantities and serve for defense against diseases and pests, as growth regulators and pigments (Tauscher, 2003).

These bioactive substances are very important in the human nutrition: even if the daily intake of phytochemicals is only ca. 1,5 g/person/day, they have a beneficial influence and are useful in prevention and treatment of human diseases. Whereas many of the secondary plant metabolites are known to be harmful in very high doses, and few, such as linamarin in cassava and solanin in potatoes are harmful in regular occurring concentrations (Heaton, 2001).

The secondary plant metabolites can be divided into four following general categories:

1. *Phenolics*

   Aromatic compounds that include flawonoids, the largest group of phenolics. Flawonoids can be sub-classified into different groups that include potentially important phytonutrients, including isoflavonoids, flavones, flawonols, anthocyanins, tannins and lignin.

2. *Terpens*

   Include the carotenoids (lutenin, lycopene), steroids, and limonoids.

3. *Alkaloids*

   A diverse group of nitrogen containing secondary compounds, synthesized primarily from amino acids which include toxic and psychoactive plant compounds such as nicotine, caffeine, cocaine, morphine, strychnine and atropine; as well as the glycoalkaloids – solanin and chaconine.

4. *Sulphur containing compounds*

   Include the glucosinolates (found in brassicas such as cabbage, kale, broccoli); allicin and other compounds from garlic and onion (Heaton, 2001).

Benefits for human health

Much of the protective effect of fruits and vegetables has been attributed to phytochemicals, which are the non-nutrient plant compounds such as the carotenoids, flavonoids, isoflavonoids and phenolic acids. Thousands of phytochemicals have been identified in foods, yet there are still many that have not been identified. Different phytochemicals have been found to possess a range of activities, which may help in protecting against chronic disease. For example, phytochemicals may inhibit cancer cell proliferation, regulate inflammatory and immune response and protect against lipid oxidation (Hollman, 1997; Liu RH, 2003). A major role of the phytochemicals is protection against oxidation. We live in a highly oxidative environment, and many processes involved in metabolism may result in the production of more oxidants. Humans, and all animals, have complex antioxidant defense systems, but they are not perfect and oxidative damage will occur. Both cardiovascular disease and cancer are thought to be particularly the results of oxidative stress, which can lead to damage of the larger biomolecules, such as DNA, lipids, and proteins. It has been estimated that there are 10.000 oxidative hits to DNA per cell per day in humans (Ames, 1993).

A major class of phytochemicals found commonly in fruits and vegetables are the flavonoids. Apples are a very significant source of flavonoids in people's diets in the US and in Europe. In the United States, 22% of the phenolics consumed from fruits are from apples, making them the largest source of phenolics (Vinson, 2001). In Finland, apples and onions are main sources of dietary flavonoids, while in the Netherlands apples rank third behind tea and onions as top sources of flavonoids (Hertog, 1993; Knekt, 1997). In a Finnish study of approximately 10.000 people, flavonoid intake was associated with a lower total mortality (Knekt, 2002). Apples were one of the main sources of dietary flavonoids that showed the strongest associations with decreased mortality.

Materials and methods

Total phenolic content: The contents of total polyphenols have been measured in the laboratory of the Department of Chemistry and Agricultural Biotechnology at the Faculty of Agriculture of the University of Pisa. The samples came from harvest, 2005 and 2006. The grounded samples have been extracted in methanol, after centrifugation at 2500 g for min, the supernatants were pooled and stored at -40℃ until use. The total phenolic content of each extract was determined in accordance with the method described in Dewnato et al. (2002). The appropriate dilutions of extracts were oxidized with Folin – Ciocalteau reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 90 minutes. The total phenolic content was expressed in gallic acid equivalents.

Lutein and zeaxanthin content: The applied method is described in Werries (2005, 2007). The grounded samples were mixed with 8'-beta-apo-carotenal and extracted tree times with methanol/tetrahydrofuran in an ultrasonic bath. The extracts with an addition of 10% NaCl were diluted with water and extracted with hexan/petrolether in a liquid-liquid extraction. The hexan/petrolether phase was pooled and concentrated in a mixture of methanol/tetrahydrofural and chromatographic solvents in a rotary evaporator. The solution was filtrated and used for the HPLC measurements.

The isocratic detection of carotenoids was performed on a C18 column (Grace-Vydac 201TP54). The column temperature was 25℃ and the applied solvents were methanol:acetonitril:2-propanol (54:44:2). The detection of the eluted carotenoids was a photometric one and was performed on a Diode-Array-Detector. A constant spectrum of λ=250 nm to 500 nm was registered while the detection was at 450 nm. Carotenoid concentrations were extrapolated from pure carotenoid standard curves.

The analysis was performed at the University Kassel / Germany. The samples were coded and treated as blind samples.

Results

Some studies highlight that the organic system produces a higher synthesis of secondary plant metabolites in confront to the conventional system because of the major exposure to pests and diseases. This is indicated for wheat too from some researches of the INRAN. In our case, the results of the analysis of total phenolic content, limited only to two years of experimentation, did not show significant differences between the two agricultural management systems. In effect in 2005 for durum wheat and in 2006 for common wheat, a very limited variation in favour of organic system was observed.

	Total phenolic content (gallic acid equivalents mg g $^{-1}$), *Triticum durum*		
	2005	2006	mean
conventional	0.37	0.33	0.35
organic	0.43	0.32	0.38
significance	ns	ns	ns
mean	0.40	0.33	*

Table 18: Effect of cropping system on total phenolic content in durum wheat

	Total phenolic content (gallic acid equivalents mg g $^{-1}$), *Triticum aestivum*		
	2005	2006	mean
conventional	0.51	0.41	0.46
organic	0.49	0.43	0.46
significance	ns	ns	Ns
mean	0.50	0.42	*

Table 19: Effect of cropping system on total phenolic content in common wheat

The two wheat species showed a difference in lutein content that was higher than expected with about 30% more in durum wheat than in the common wheat, while both species were similar in zeaxantin content. The cropping system did not seem to have any effect on lutein content, while the differences in terms of zeaxantin between the two systems are significant only in durum wheat.

There was a tendency in *Triticum aestivum* samples for a slight higher content of lutein in the organically grown samples (in two years for two replicates). The organic *Triticum durum* samples showed slight lower concentrations of carotenoids (lutein and zeaxanthin) in the second year. The contents of carotenoids differed moreover within the bocks for conventional samples, whereas for organic samples the differences were much lower. The contents of carotenoids were in both years similar and the differences between the blocks were more or less the same as for the cultivation systems.

	Lutein content (µg g^{-1}), *Triticum durum*		
	2005	2006	mean
conventional	3.30	3.43	3.37
organic	3.23	2.87	3.05
significance	ns	ns	Ns
mean	3.27	3.15	ns

Table 20: Effect of cropping system on lutein content in durum wheat

	Lutein content (µg g^{-1}), *Triticum aestivum*		
	2005	2006	mean
conventional	0.98	1.03	1.00
organic	1.03	1.17	1.10
significance	Ns	ns	Ns
mean	1.00	1.10	ns

Table 21: Effect of cropping system on lutein content in common wheat

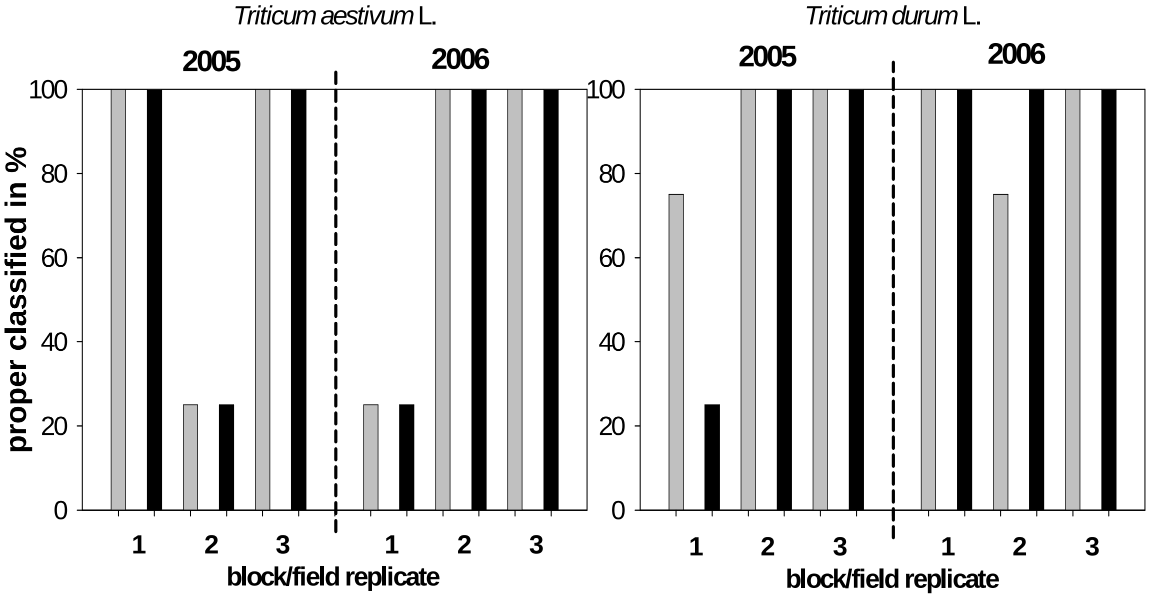
	Zeaxanthin content (µg g^{-1}), *Triticum durum*		
	2005	2006	mean
conventional	0.25	0.32	0.29
organic	0.21	0.26	0.24
significance	ns	ns	*
mean	0.23	0.29	ns

Table 22: Effect of cropping system on zeaxanthin content in durum wheat

	Zeaxanthin content (µg g^{-1}), *Triticum aestivum*		
	2005	2006	mean
conventional	0.20	0.24	0.22
organic	0.19	0.25	0.22
significance	Ns	ns	Ns
mean	0.20	0.24	**

Table 23: Effect of cropping system on zeaxanthin content in common wheat

The discriminant analysis was performed for the two species separately. For Triticum aestivum only the contents of lutein and for Triticum durum the contents of lutein and zeaxanthin were taken in consideration. The results showed that the write classification of the samples was possible for Triticum aestivum in 70,4% and for *Triticum durum* in 80,9%.



Graphic 8: Classification of the MASCOT wheat samples according to the farming system (organic: grey, conventional: black). The bars are showing the percentage of proper classified sample replicates per field replicate/block.

Conclusions

The effect of the cropping system on the total phenolic content of wheat grain is not so clear by the data of the MASCOT experiment, even if a moderate increase is supposable in the organic system. This parameter needs to be more carefully investigated.

The differences in lutein content of the two cropping systems were very small and statistically unappreciable. The two species however differed predictably in terms of lutein content: durum wheat containing 30% more than the common wheat. In both years there also was a higher content of zeaxanthin in the conventional samples of *Triticum durum*, but no difference for *Triticum aestivum* was found. The discriminant analysis classified correctly 70,4% and 80% of the *Triticum aestivum* and *Triticum durum* samples respectively, however the correct classification of all samples was not possible.

3.3. Experiment II - Sensory analysis

3.3.1. Sensory analysis of the attributes of organic and conventional bread

Background

Among the claims made about organic growing methods is that they produce more flavourful (‘better tasting’) products. In the recent years a number of studies was undertaken (Schutz, 1976; Gieland, 2006) to determine the validity of this claim.

Sensory Analysis is "the science of using human sense organs for testing and measuring purposes" (definition according to DIN 10950-1). All sensory evaluation techniques can be broadly classified into three categories:

- Discrimination tests, which allow a determination of the presence of differences;
- Descriptive analysis techniques, which use trained panels to describe the nature of, and quantify, any differences that may be present;
- Preference/acceptability measurements that reflect relative degrees of liking. (Bourn, 2002)

The sensory analysis is gaining as a method for discrimination of organically and conventionally grown foods recognition. The senses of trained people (schooled panel according to DIN/ISO) become a scientific evaluation tool of the product quality in this analysis. The sensory profile of the analysed food item is perceived and described, so that differences in quality can be detected (Rahmann, 2006). In the sensory analysis the panellists are working with the whole, undivided product, therefore the sensory can be classified to the holistic food quality analysis methods.

The aim of this study was to give preliminary results of an informal organoleptic satisfaction test on bread derived from the winter wheat coming from the organic and conventional cropping systems of the MASCOT long term experiment. The particularity of this test, no common to others of the same type, is that the variety compared were the same and the variation between the two breads are represented only by the cropping system (Mazzoncini et al., 2007).

Materials and methods

Wheat flour was obtained from the *Triticum aestivum* samples grown in 2005 by stone milling the grain in a water driven mill and then sifting it through sieves, separating out only a part of the seed coats and not eliminating the germ. Bread loafs were baked in a commercial bakery. The test on bread loafs was performed by semi-trained panellists represented by the CIRAA staff formed by 44 people. The test included organoleptic analyses related to flavour, fragrance, crust and crumb consistency. Uniform sized samples were pre-coded and presented to the panellists; clean water was provided to the panellists to rinse their mouths between samples tasting. An evaluation form was provided to each panellist to record his or her judgement. The following characteristics were evaluated: crust consistency (crisp, intermediate, hard); crumb consistency (soft, firm, hard); flavour (tasty, acceptable, unpleasant); fragrance (pleasant, neutral, unpleasant); overall evaluation (excellent, good, acceptable, poor).

Results

27% and 73% of the panellists savoured conventional and organic bread respectively. Conventional bread appeared to be characterized by having a crisp crust (54%), a soft crumb (72%), an acceptable flavour (56%) and a pleasant (60%) fragrance. 60% of the panellists gave it an overall evaluation of good and 40%, acceptable. The crust of organic bread was judged crisp by 41% of the panellists and the crumb as firm by 45%. Most panellists found it tasty (91%) and pleasantly fragrant (74%) and gave it a good overall evaluation (55%), but all the other overall evaluation values were given too (19% excellent, 16% acceptable, 10% poor). It should be pointed out that 36% of the panellists found that organic bread had a hard crust and 19% a hard crumb, while only few people expressed these opinions about conventional flour bread. About 90% of the panellists preferring organic bread found it tasty, referring to an “old- time”, “a grassy” or a “more authentic” flavour.

	Overall evaluation			
	excellent	good	acceptable	poor
Conventional	-	60	40	-
Organic	19	55	16	10

Table 24: Panellists overall bread evaluation (%)

Conclusions

The results show that the organic bread was judged by the panellist to have a harder crust and crumb in comparison to the conventional bread. This finding is also referred in some other sensory studies on organoleptic proprieties of bread baked out of organic and conventional flour. Hadlung et al. (1998) found that organic bread was characterised by a higher dryness and lower elasticity as compared with the conventional one. A lower elasticity and juiciness for organic bread was also referred by Kihlberg (2004) and Annett et al. (2007) who found the organic bread to have a denser texture and smaller air cells in the appearance of the crumb when compared with conventional bread. The higher hardness of the organic bread is expected to be caused by the lower protein content of the organic flour and a lower elasticity of dough (see experiment 1), thus the flour composition has a strong impact on sensory attributes of bread (Kihlberg, 2004). However most panellists found the organic bread to be “tasty”.

3.3.2. Preference test of organic and conventional bread

Background

Investigations of the sensory quality of bread produced with wheat from alternative farming systems and of consumer acceptance of these products are not well known, but are decisive for acceptance and purchase decisions. For consumers, the sensory quality, especially “taste”, is reported to be the most important purchase criterion (Kihlberg, 2004) and 41% of consumers give taste as a major reason for purchasing the organic product (MORI, 2001).

Although the differences in taste are difficult to confirm, there are some findings in controlled studies, which refer differences in taste in organic and conventional products in favour for the organic products (Rembiałkowska, 2000; Dlouhy, 1981; Weibel et al., 2000). There are also studies, where no significant difference between organic and conventional products was found (Basker, 1992).

Materials and methods

Wheat flour was obtained from the *Triticum aestivum* samples grown in 2005 by stone milling the grain in a water driven mill and then sifting it through sieves, separating out only a part of the seed coats and not eliminating the germ. Bread loafs were baked in a commercial bakery. The test on bread loafs was performed by customers of the same commercial bakery that baked the bread. The number of interviewed people was 157, whose average age was 51. Uniform sized samples were pre-coded and presented to the panellists; clean water was provided to the panellists to rinse their mouths between samples tasting. An evaluation form was provided to each panellist to record his or her judgement.

Panellists were asked to answer to the following questions: “Which bread do you prefer?” and “Why do you prefer it?” (flavour, flavour and consistency, consistency, uncertain).

Results

Organic bread was preferred both by the women (61%) and the men (57%) who were interviewed; 6% of women and 12% of men did not express any reference. Customers who chose organic bread stated that they preferred it (table 5) for its flavour (40%), flavour and consistency (34%), consistency (16%) and 10% were uncertain.

The results of the study showed that flavour had the strongest influence amongst other characteristics in guiding the customer's preferences.

	Flavour	Flavour and consistency	Consistency	Uncertain
Conventional bread	43	24	25	8
Organic bread	40	34	16	10

Table 25: Panellists answers (%) to the question: *Why do you prefer the bread you chose?*

Conclusions

The results confirm that organic bread can be largely appreciated by a big part of customers of a commercial bakery who normally are not used to taste organic bread. Flavour seems to have a leading role in the customers choice even if differences to the common product are detected.

3.4. Experiment III - The biocrystallization method as a tool to asses wheat quality from organic and conventional cropping systems.

3.4.1. Methodology, used equipment, and the biocrystallization process

The set up of a specific methodology for wheat at CIRAA laboratories represent an important part of the present research work. The method was progressively adapted according to the observed results until it reached the final codification.

3.4.1.1. Sample preparation for wheat

In all the experimental work the sample preparation was always performed in accordance to the following sample preparation procedure.
15 g of cleaned wheat kernels are grounded in a GAGGIA mill (LAMA/A, Type ML) for three minutes. After thoroughly mixing the ground material, 10 g is used to produce a 10% aqueous extract in 90 ml ultra-filtrated, deionised water, with centrifugation at 160 rpm for 45 minutes at room temperature. The extract is left for 15 minutes and than filtrated by means of a IF 5A filter. The crystallization solutions are produced in ultra-filtrated deionised water with a fixed or variable amount of copper chloride per plate. The crystallization solution is placed on Petri dishes and left for ca. 24 hours in a *GIRARD SUDRON* crystallization chamber (see picture 4). The formed patterns are scanned on an EPSON scanner. The grey level analysis is performed on scanned patterns and the visual analysis – on printed patterns.

3.4.1.2. The crystallisation process

The crystallization process takes place in a closed crystallization chamber, in constant temperature of 30℃ inside the chamber and constant temperature of 25℃ outside the chamber in order to avoid air turbulences around the plates and to allow a diffusive transport of heat and humidity during evaporation and crystallization. The climatic conditions inside the chamber are controlled (temperature) and measured (temperature and relative humidity) continuously.

The crystallization process consists of two phases: the evaporation and the crystallization.

Once the plates are placed in the chamber the relative humidity grows quickly because of the beginning of the evaporation process. The evaporation slows down after a rapid beginning, until the crystallization solution saturates. Then it stops with the appearance of the first crystals, which are usually placed in the centre of the plate. The appearance of the first crystals indicates the initiation of the crystallization phase, which goes on until the whole plate is covered by the crystal structure. The relative humidity decreases during this phase and finally, at the end of the crystallization phase, reaches the value it had before the plates were placed in the chamber. The duration of the evaporation phase influences the duration of the following crystallization. The longer the evaporation the shorter the crystallization and vice versa.

3.4.1.3. The crystallization chamber

The crystallization chamber applied at the CIRAA laboratory is a cube-like chamber (0,90 x 0,70 x 0,70m) constructed from plywood elements. The front side has a built-in glass window and the top side is provided with holes which enable air circulation during the crystallization. The chamber is heated electrically with the use of bulbs placed under a glass table, on which the pictures crystallize. This chamber is capable of producing 16-20 pictures simultaneously. The temperature is regulated by means of a thermostat placed above the table in opposition to the entrance door. The chamber is also equipped with a relative humidity sensor. The little room, inside of which the chamber is placed, has an air conditioning system. This assures constant climatic conditions outside the chamber and prevents incidental influences on the crystallization conditions inside the chamber.

Picture 3: The GIRARD SUDRON crystallization chamber.

3.4.1.4. The visual evaluation of the crystal patterns

The visual evaluation of the patterns was performed by the author of this thesis. Whole patterns and parts of patterns containing characteristic structural elements were compared and described. The description of the patterns was based on wheat criteria, described and developed by the partners of the “Triangle” – a collaboration of three biocrystallization laboratories: the University of Kassel / Germany, the Luis Bolk Institute / Holland, and the BRAD Institute / Denmark. These criteria regard both structural elements and general pattern characteristics, which can appear in crystallograms of wheat extracts. The features described in the visual criteria can appear in the patterns with different degrees of intensity. The main scope of the introduction of these criteria to this thesis is the vocabulary standardization, which is indispensable for a clear description of forms and structures.

The criteria for the visual evaluation of wheat patterns:

Integration: The different form-elements are more or less related to one-other. The pattern can more or less be sensed as a "wholeness".

Centre coordination: from the centre a more or less ordering function is working.

Irradiation (Durchstrahlung): Indicates how far the head branches reach from the centre towards the peripheral zone and how powerful they are formed.

Clear stems: The stems can be more or less separated.

Mobility (Beweglichkeit): indicates the movement in the needle structure of the pattern. The needle structure can be more or less stiff or curved.

Fullness with side needles: The pattern can be more or less naked or full with side needles.

Substance spirals: Substance spirals are rings or spirals visible in the structure of the patterns. The patterns can be more or less full with substance spirals.

Oblique needles (Quernadeln): Individual needles or small branches, which deviate from the stems by a bigger angle than the rest of the side needles.

Dense radial formations: In the central and middle zone a dense, radial, felt like or interwoven formation can appear.

3.4.1.5. The computer based evaluation powered by the *Image J* software

The scanned patterns, besides visual evaluation, were also analyzed by use of the *Image J* computer software for image processing and analysis (Reinking, 2007).

After the crystallization the patterns were scanned by 3300x3300 pixels and analysed for the grey level distribution by the use of the *Image J* software. The mean values of the grey level distribution were measured within ten ROI's (Regions of Interest) of circular shape, with the centres placed in the geometrical centre of the picture and diameters of different length. The diameter of ROI 10 was equal with the picture's diameter, whereas the diameters of ROI 9 to 1 were equal with 90% to 10% of ROI's 10 diameter respectively. This analysis allows to see differences in the density of crystal structures of the patterns. The grey level values (from 0=black to 255=white) of all points of the scanned picture are calculated. The lighter areas, which are those covered by crystals, have higher values of grey levels than the uncovered areas. Therefore the denser the crystal structure, the higher the mean value of grey levels. The mean values of the grey level distribution calculated within ten ROI’s of patterns crystallized out of the organically grown samples were compared with the grey values of patterns crystallized out of the conventional samples. The results were presented in form of graphs and analysed statistically by use of the one way ANOVA for randomized blocks.

The results of this analysis are regarding only the degree, in which the picture’s are covered by crystal structures and do not regard their gestalt (design created by crystal structures).

3.4.2. Monitoring of the relative humidity conditions and crystallization time

Background

When producing biocrystallization pictures from crop and product samples the numerous organic compounds present in the juice / extract will create very complex conditions in the evaporating solution. These include the colloidal and hydrophilic properties of the additives, the particle size distribution of the additives, as well as the overall viscosity, density and the pH of the solution (Barth, 1997). Thus producing reproducible crystallizations presupposes ideally a strict control of physical conditions – temperature and relative humidity.

This experiment was carried out for a better understanding of he the crystallization process and the variations induced by the addition of a vegetable extract.

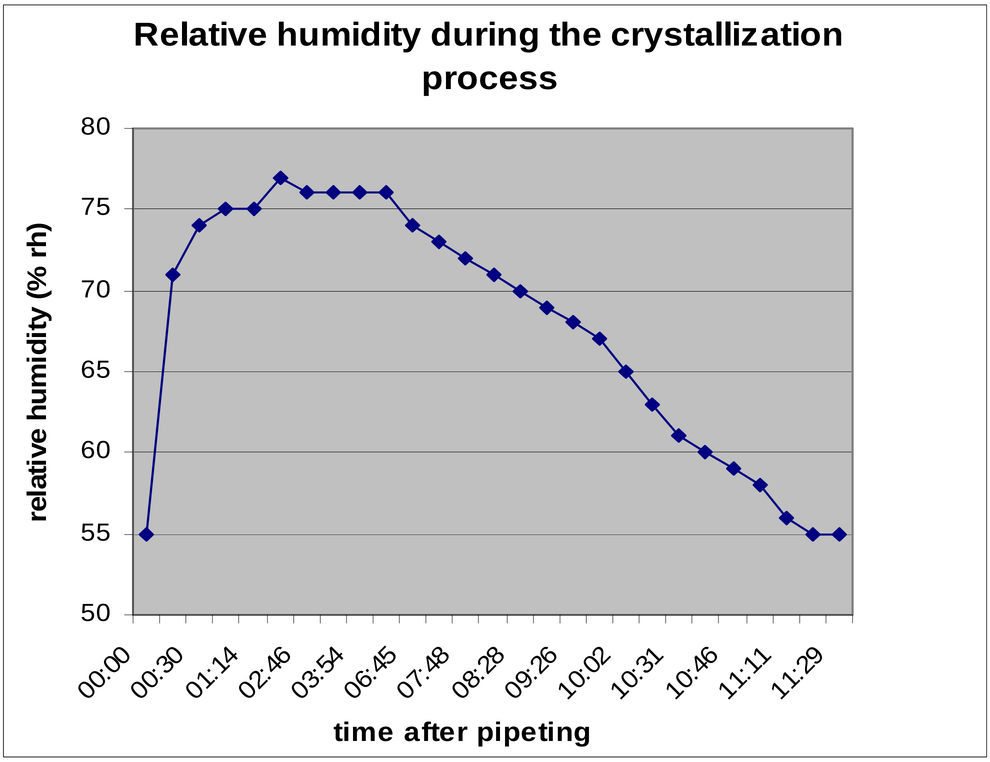
Materials and methods

The experiment consisted in the collection of the relative humidity values all over the crystallization process of three different crystallization solutions, each one on 6 plates (18 pictures in total) to illustrate the relative humidity fluctuations during the crystallization and the relation between the duration of the two phases: the evaporation and crystallization.

Three different crystallization solutions of different densities were prepared: the first contained only cupper chloride and water, without any organic additive; the second one contained a fresh extract of *Triticum aestivum* grain in a mixing ratio of 90/70 (0.9 mg of 10% $CuCl_2$ and 0.7 mg of the extract per plate) and the third one contained a ten days old *Triticum aestivum* extract in the same mixing ratio. Each crystallization solution was pipeted on six plates randomly placed in the chamber and crystallized. The values of relative humidity were collected every 15 minutes during the crystallization. In addition the time of the crystal's first appearance on each plate (end of the evaporation phase) and the time when the whole plate was covered by crystal structures (end of the crystallization phase) were noted.

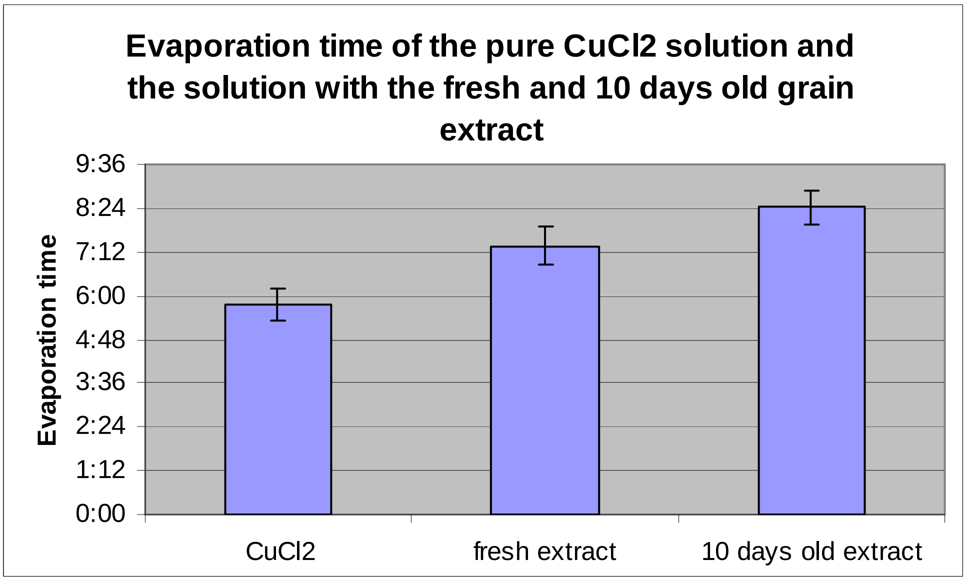
Results

At the beginning of the experiment, before pipeting, the relative humidity took out 55% rh. After the solution was pipeted, the relative humidity began to rise and after two hours reached the maximum value of ca. 85% rh, which remained constant for further four hours, and than began to decrease. About three hours after pipeting first crystals were formed on all plates. The crystallization went on for further nine hours until the last plate finished to crystallize. The relative humidity returned to the value of 55% rh, as it was before the pipeting. The graph below shows the curve of relative humidity values measured in this experiment.

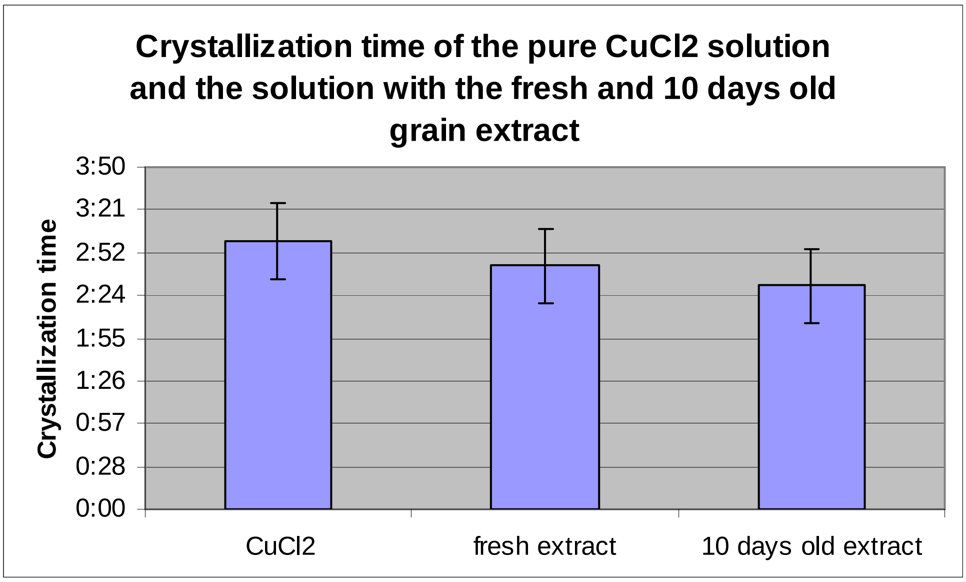


Graphic 9: Relative humidity during the crystallization process.

The results showed, in regard to the evaporation and crystallization time of the different solutions, that the three crystallization solutions (the solution with the addition of fresh grain extract, the solution with the addition of the ten days old grain extract and the pure cupper chloride solution) varied in the durations of their evaporation and crystallization phases.



Graphic 10: Evaporation time.



Graphic 11: Crystallization time.

The plates with the pure $CuCl_2$ solution had the shortest average evaporation time of 5:45 hours. The plates with the addition of the fresh extract evaporated on average in 7:22 hours, and the plates with the addition of the 10 days old extract - in 8:25 hours. The tendency in the duration of the following crystallization times of these three solutions was inversely proportional to their evaporation times. The plates with the pure $CuCl_2$ solution crystallized on average in 3 hours, and the plates with the solutions with addition of the fresh and 10 days old grain extracts crystallized respectively in 2:44 and 2:30 hours. The negative correlation between the evaporation and crystallization times of these three solutions amounts to R= -0,99. The different evaporation times most probably depended on the different densities of the three crystallization solutions.

Conclusions

The results showed that there is a clear negative correlation between the evaporation and crystallisation time of the solutions. In the here described experiment three solutions with different densities have been used; but it has been found that also during the crystallization of one solution, plates which evaporated faster had a longer crystallization time, and vice versa (Busscher, 2003). The crystallization time of patterns influences the patterns diameter (Irradiation / *Durchstrahlung*) (Busscher, 2003). Therefore it is important to compare pictures with more or less the same crystallization time. No significant differences in the crystallisation time of the same solutions have been observed in the chamber used in the laboratory of the University of Pisa.

3.4.3. Matrix assessment

Background

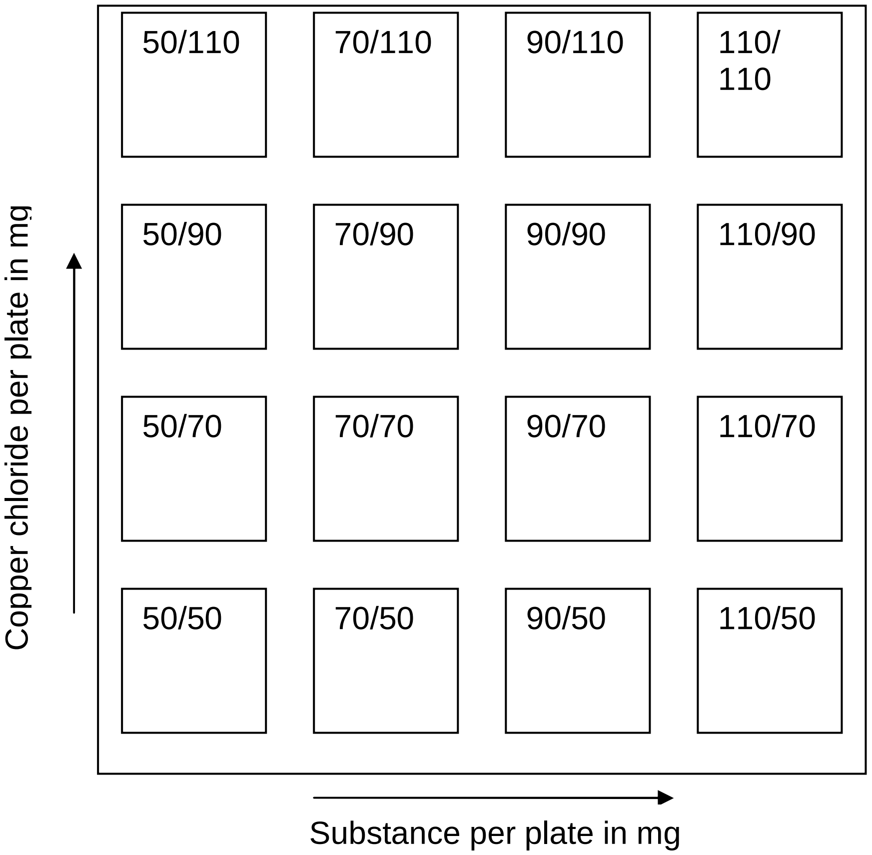
As resulted from the previous experiment, the concentration of the solution seems to have an important influence on the crystallization process and on some morphological features.

The determination of a suitable concentration between the sample in question and the reagent copper chloride is an essential step in the biocrystallization analysis. The patterns made out of solutions with different concentrations show different pictomorphological features: an excess of cupper chloride is causing the creation of dense radial formations, the stems are stiff and the pictures have less or no side needles. The key presence of the organic extract provokes the appearance of many side needles as well as concentric formations called by some researchers "substance spirals” - the pictures seem to be bushy and no clear stems are visible. An optimal concentration between the organic extract and cupper chloride allows the full development of features in the picture, which can be used as a quality indicator. Pictures with the optimal concentration are normally characterized by a good visible form and are specific for the analysed sample.

The construction of a concentration matrix is a procedure for determining such an optimal concentration. Graphically a concentration matrix is arranged with the concentrations of the sample and the reagent on the horizontal and vertical axes respectively (see picture below). A matrix is generally made up from four to five concentrations of both the sample and the reagent, thereby yielding a total of 16, 20 or 25 concentration combinations (Andersen et al., 2003).

Because of the variation of features appearing in pictures in accordance to different concentrations of copper chloride and substance applied during crystallization, it is possible to compare pictures made up only of the same concentrations.

The goal of the experiment was to determine the optimal concentration of substance and copper chloride per plate for the organically and conventionally grown *Triticum durum* and *Triticum aestivum* samples. The intention was, if possible, to determine one suitable concentration for all samples, in order to be able to compare the two species. Examination and comparison of picture’s structures of the two species in question in varying concentrations of copper chloride and substance.



50/110	70/110	90/110	110/ 110
50/90	70/90	90/90	110/90
50/70	70/70	90/70	110/70
50/50	70/50	90/50	110/50

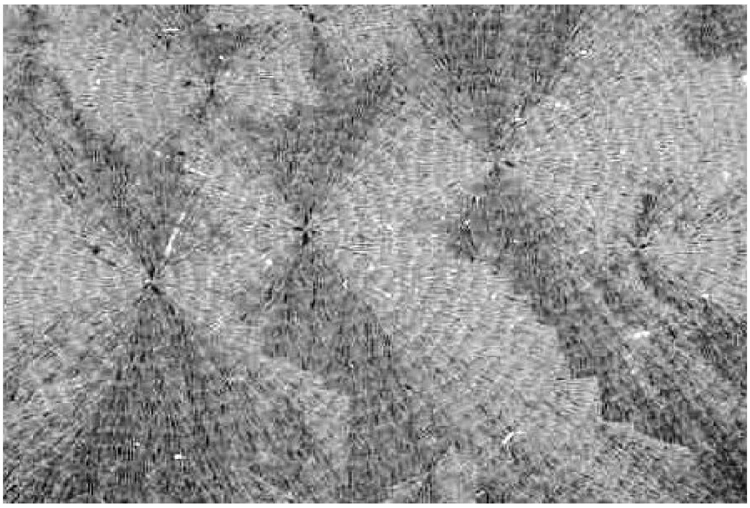
Picture 4: Concentration matrix.

Materials and methods

A total of four concentration matrixes was prepared for the organically and conventionally grown *Triticum aestivum* and *Triticum durum* samples. The three field replicates for each specie and cultivation system were mixed together in order to receive one sample (four samples in total), which then was prepared in accordance to the sample preparation procedure. The following concentrations of substance and copper chloride per plate applied was used in the matrixes: 50/50, 70/50, 90/50, 110/50, 50/70, 70/70, 90/70, 110/70, 50/90, 70/90, 90/90, 110/90, 50/110, 70/110, 90/110, 110/110.

Results

The matrixes showed the whole spectrum of patterns of different concentrations (see Appendix no. 1). In the **right low corner** appear substance dominated pictures (highest concentration of substance, lowest concentration of copper chloride). These pictures basically show no defined forms. There is only a very dense needle structure, which covers the whole plate. This needle structure can be made up of two different kinds of needles: light needles and shadow needles (see picture 6). Also substance spirals can appear. The pictures with high substance concentration are often multi centred.



Picture 5: A substance dominated picture, concentration 110/50.

In the **left upper corner** of the matrix the pictures are copper chloride dominated (the highest concentration of copper chloride and the lowest concentration of substance). The typical features for these patterns are dense radial formations and huge lemniscates (i.e. a form shaped as an eight, placed on both sides of the centre) (see picture 7). There are basically no needles on the stems.

Picture 6: A cupper chloride dominated picture, concentration 50/110.

Pictures with visible stems and defined forms appear on the line **between the left lower corner and the right upper corner** of the matrix. These pictures show an equilibrium between the copper chloride and substance (see picture 8).

Picture 7: A picture with well formed structures, concentration 70/90.

The matrixes of conventionally and organically grown samples differed from each other for both species *Triticum durum* and *Triticum aestivum*. There were more substance dominated pictures in the matrixes created out from organic samples and the pictures with the equilibrate concentrations of substance and copper chloride appeared in the organic matrixes with slightly higher concentrations of copper chloride than in the conventional matrixes.

The results of the matrix procedure permit to identify the most suitable ratio between the concentration of cupper chloride and the extract in order to produce pictures with clear features, which allow the differentiation between the two cropping systems.

The chosen concentration for the *Triticum durum* and *Triticum aestivum* matrixes was the same for both species because of only little differences in the optimal concentration and amounted on 70/90.

The *Triticum durum* pictures were slightly more substance dominated. This characteristic of the *Triticum durum* pictures could be caused for instance by different composition of the aqueous extract and gluten and, in turn, by different behaviour of the extract during the filtration. This question needs further investigation.

Conclusions

The matrix procedure is an important preliminary experiment that allows the choice of a suitable concentration for the samples in question as also permits to gain first information about the samples and their behaviour during crystallization. In contrast to concentration series, where different sample amounts are applied by the same amount of reagent, the two dimensional arrangement of the matrix permits to examine different amounts of both sample and reagent contemporary (Andersen, 2003).

3.4.4. The biocrystallization of organically and conventionally grown *Triticum durum* and *Triticum aestivum* samples

Background

The scope of the application of the biocrystallization method as a method of analysis for the discrimination of the organic and conventional MASCOT wheat samples was the enrichment of the common analysis results by the holistic understanding, which is not based on the quantity of several substances in the samples, but on the view on the whole, undivided product.

Materials and methods

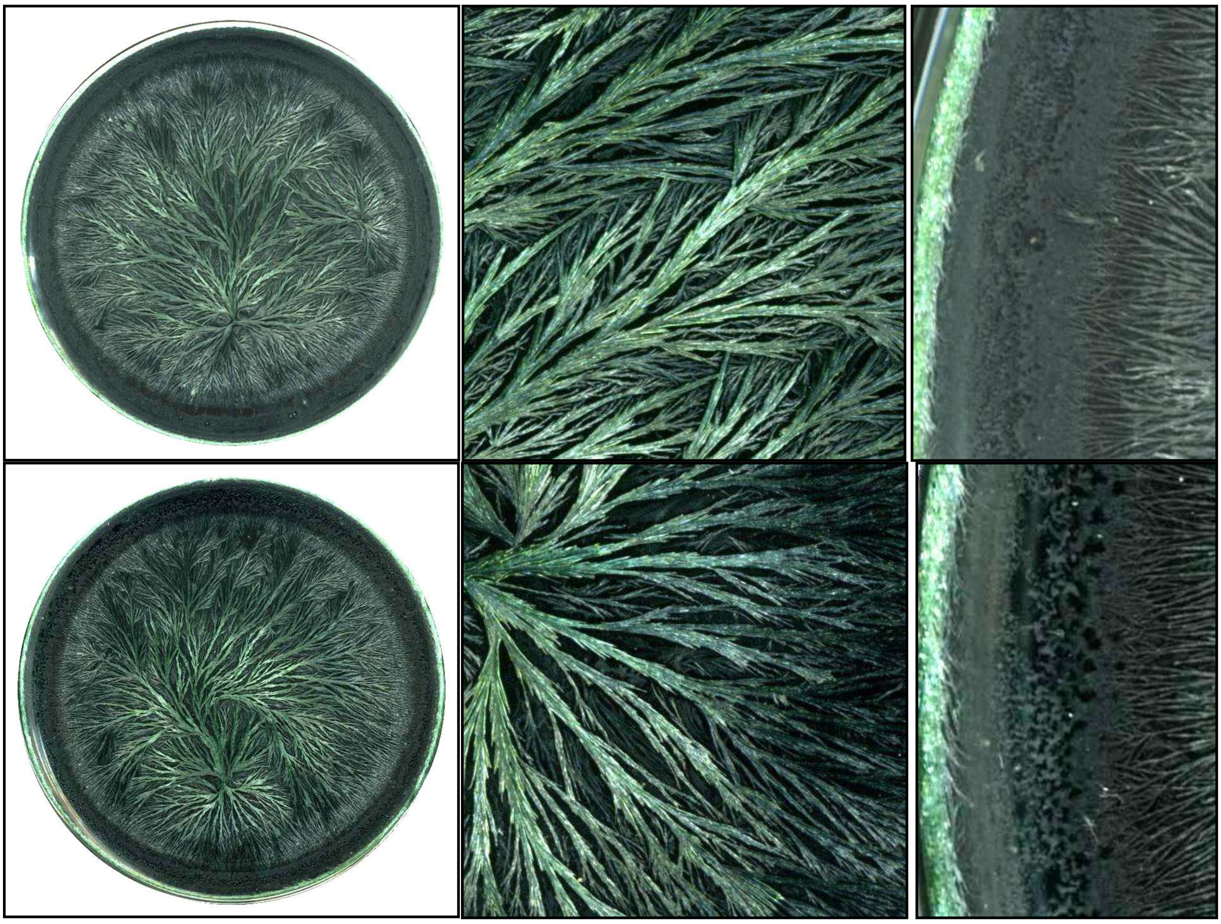
A total of 24 samples (organic and conventional *Triticum aestivum* and *Triticum durum* samples coming from harvests 2005 and 2006) was prepared in accordance to the laboratory procedure described in *Sample Preparation for Wheat Samples* (see chapter 3.4.1.). Out of each sample three patterns were crystallized. In accordance with the results of the matrix experiment the mixing ratio between the 10% wheat extract and 10% cupper chloride solution was 70/90. The resulting patterns were scanned and evaluated with the *Image J* software for the grey level distribution. The visual evaluation of the pictures was based on the comparison of whole pictures, parts of the middle zone and parts of the border zone of the pictures.

The visual evaluation of the resulting patterns consisted in the observation and comparison of features and structures appearing in the crystal patterns. The attention was focused on the overall character, the branches of the middle zone and the crystal structures of the border zone. The observed features were described using the crystallization criteria (see chapter 3.4.1.).

- Firstly the differences between the two species: *Triticum durum* and *Triticum aestivum* were analyzed and described.
- A contrast of the patterns generated out of organic and conventional grain samples was made.
- And finally a comparison of the two harvesting years was done.

Results

Examples of the resulting patterns of *Triticum aestivum* and *Triticum durum* are shown below. All patterns with examples for field replicates, samples and year notes are to be found in Appendix 2.

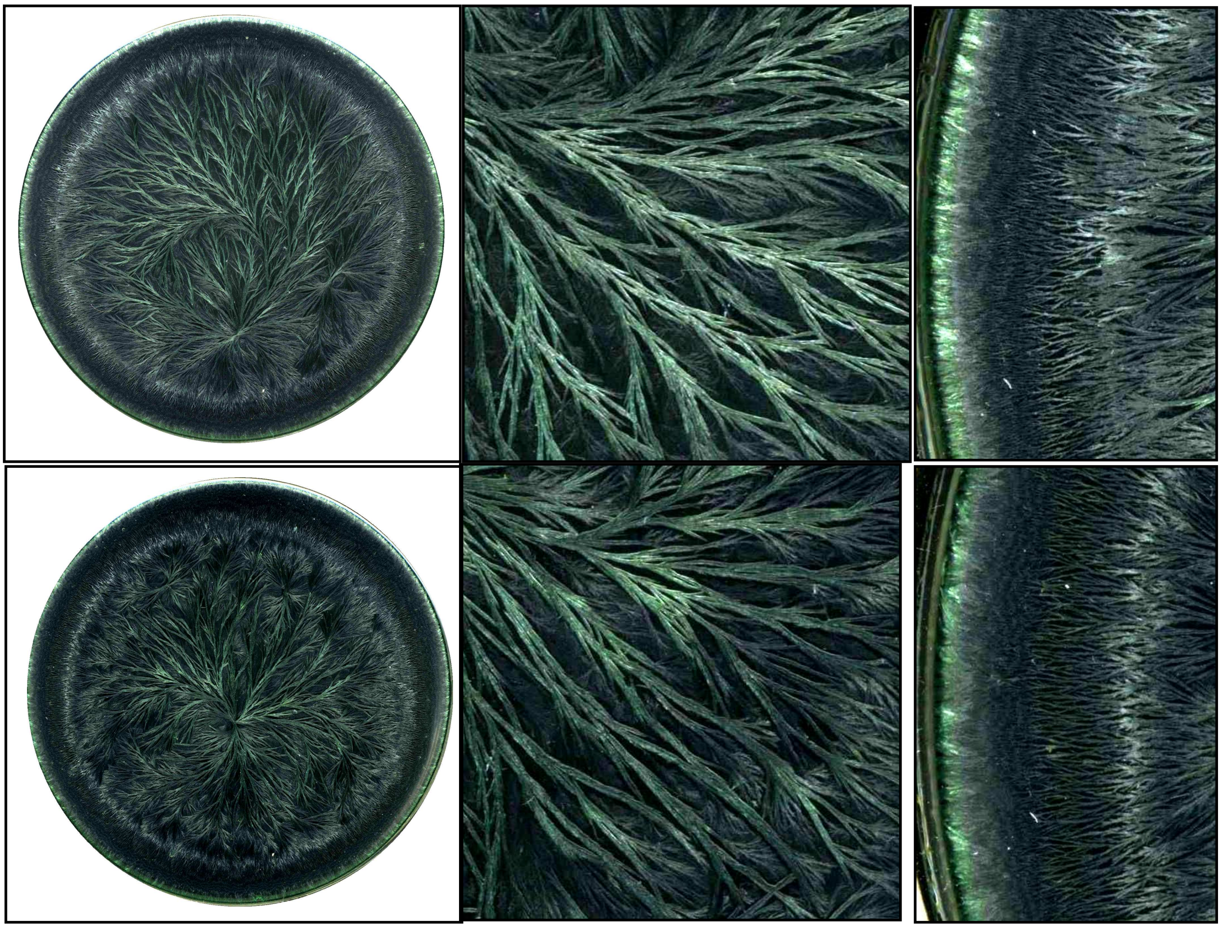


Picture 8: *Triticum aestivum* patterns, harvest 2005. From the left: whole pattern, stems from the middle zone, border zone.

<u>Differences between the two species</u>

The biocrystallization patterns generated out of *Triticum durum* and *Triticum aestivum* samples differ in their structures and overall characteristics. The main difference is the density of needles, which is much higher in the *Triticum durum* patterns. In the *Triticum aestivum* patterns the visibility of branches running from the centre of the picture out to the periphery zone is therefore better (clear stems) and single ramifications can be seen easily. The fullness of needles in the *Triticum durum* patterns “covers” the run of the stems, which in some cases can only be “guessed”. The needles appear in bundles and therefore the patterns seems to be bushy.

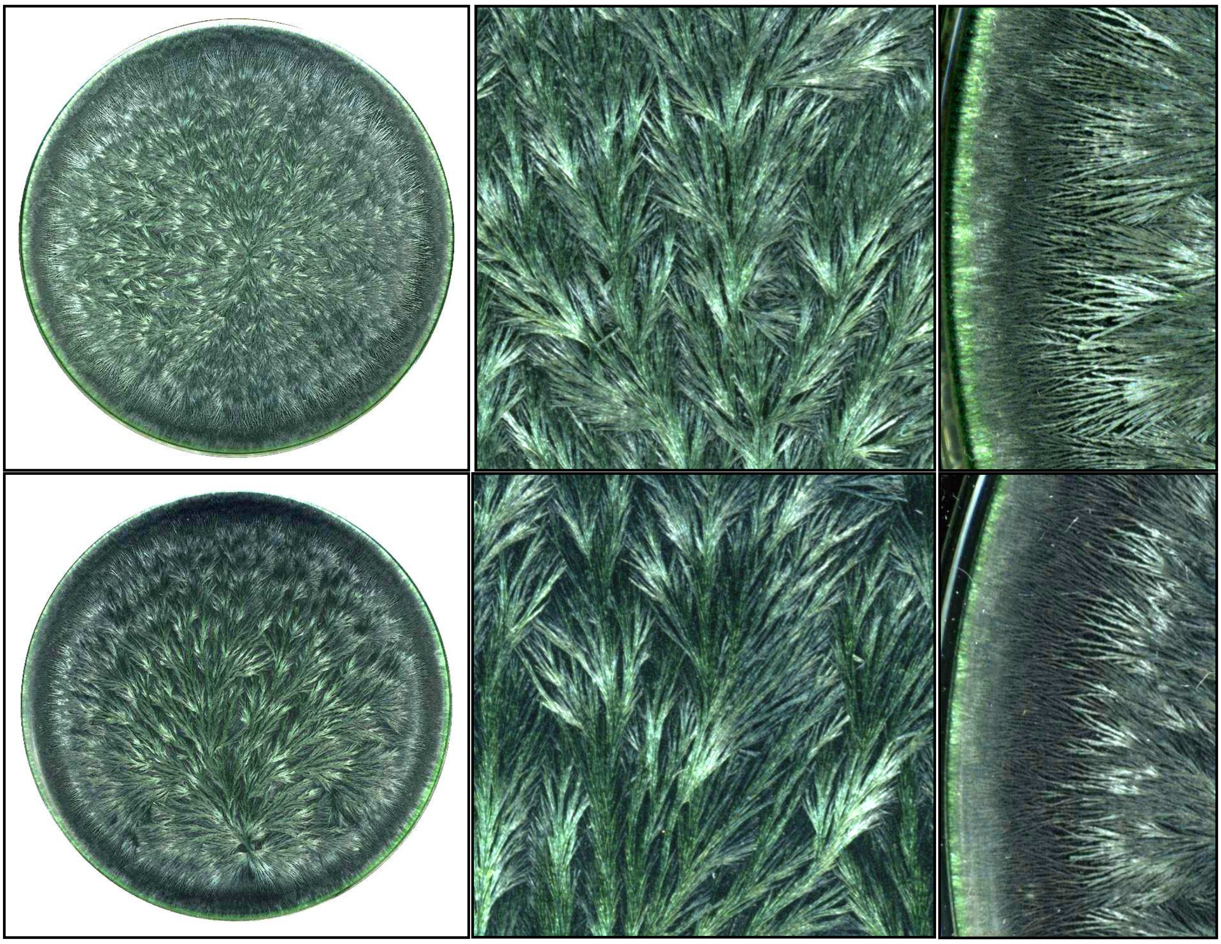
Another difference between the patterns of the two species is the presence of substance spirals in the *Triticum durum* patterns, i.e. an alternant appearance of lighter and darker needle rings placed around the picture's centre. The substance spirals can appear in different degrees of density and in different parts of the pattern. In the here analysed *Triticum durum* patterns the substance spirals are intensive and their density is rather high, moreover their appearance is not limited to a certain part of the picture, but occurs in the whole picture. This causes, that the *Triticum durum* patterns seem more homogenous in their structures than the *Triticum aestivum* patterns.



Picture 9: Triticum aestivum patterns, harvest 2006. From the left: whole pattern, stems from the middle zone, border zone.

Differences between the two cropping systems

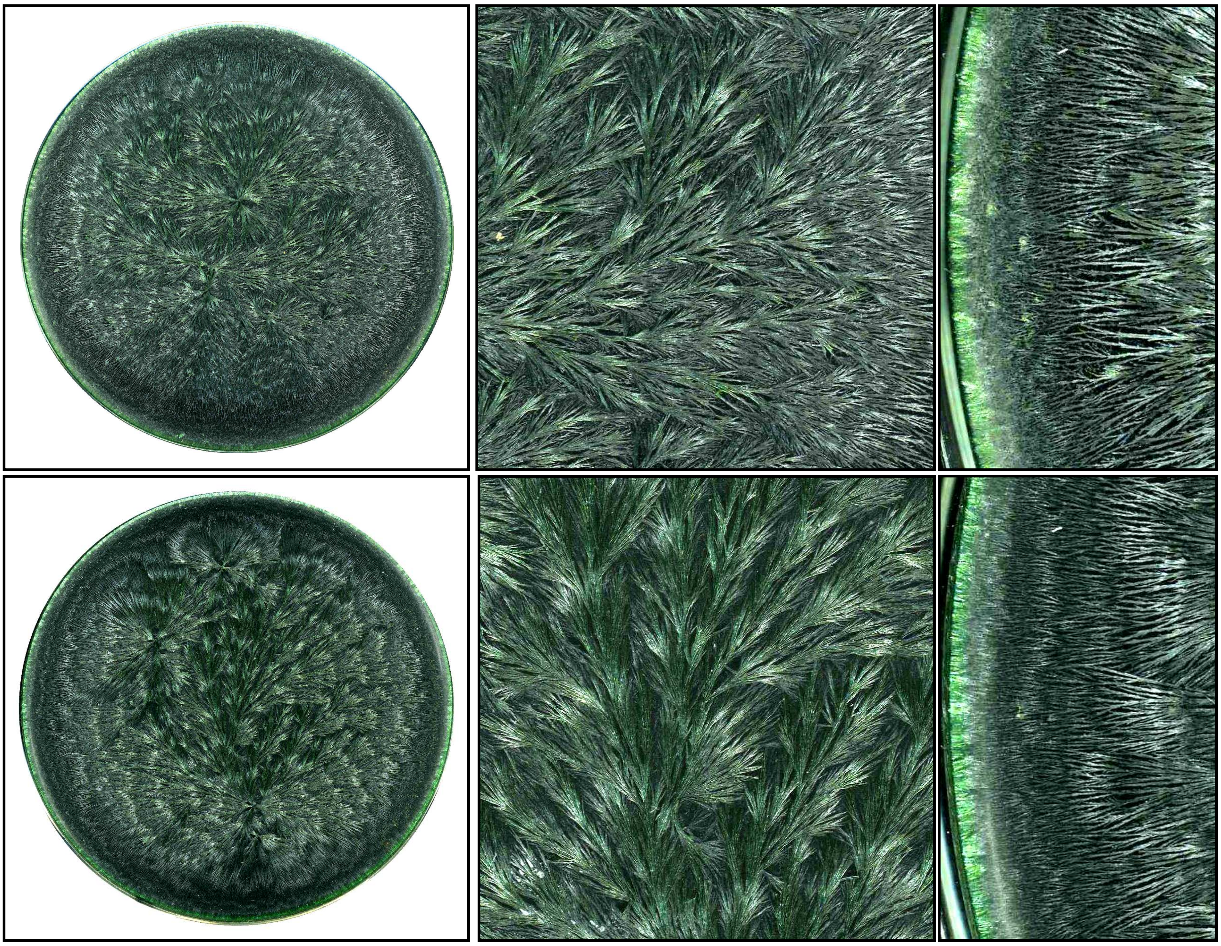
The organic and conventional grains generated patterns, which differed in their overall characteristics and some features. In the *Triticum aestivum* patterns the features: Irradiation (how far the head branches reach from the centre towards the peripheral zone and how powerful they are formed) and “Fullness with side needles” were stronger in the organic samples. The patterns of organic and conventional samples differed also in their border zone structures. The border zone in the patterns generated out of the organic samples was covered by crystals, whereas the border zone in the patterns of the conventional samples was often uncovered. The *Triticum durum* patterns showed also differences between the conventional and organic samples. The patterns of organic samples had a better “Integration” (the different form-elements are more or less related to one-other and the pattern can more or less be sensed as a “wholeness”) than the conventional samples and they had also a better “Fullness with side needles”. Also more “Substance spirals” appeared in the patterns of organic samples.



Picture 10: Triticum durum patterns, harvest 2005. From the left: whole pattern, stems from the middle zone, border zone.

Differences between the two harvesting years

There have been differences in the patterns of the two species: *Triticum aestivum* and *Triticum durum* coming from the two harvesting years 2005 and 2006. Generally the patterns of both species in question harvested in 2006 showed a denser needle structure, more substance spirals (in case of Triticum durum) and a better structured border zone. The needles of the patterns from 2006 are generally shorter and the patterns seem more bushy. Based on the results it can be claimed, that the patterns created out of the samples harvested in 2006 are more substance dominated than the patterns created out of samples harvested in 2005.



Picture 11: Triticum durum patterns, harvest 2006. From the left: whole pattern, stems from the middle zone, border zone.

Analysis of the grey level distribution

The results of the analysis of the grey level distribution confirmed the results of the visual evaluation.

In the scanned patterns the crystal structures appears lighter than the uncovered spaces. By the use of the *Image J* software the values of the grey level distribution were counted, where the value “0” (empty) means black and the value “255” (covered) means white, therefore the denser the crystal layer of the pattern, the lighter it appears, and the higher its value in the grey level distribution.

Differences between the two species

It could be also observed that the Triticum durum patterns had higher values of the grey level distribution in comparison to the *Triticum aestivum* patterns, and had in consequence a denser crystal layer than the second ones. This observation also confirms the results of the visual evaluation.

Differences between the two cropping systems

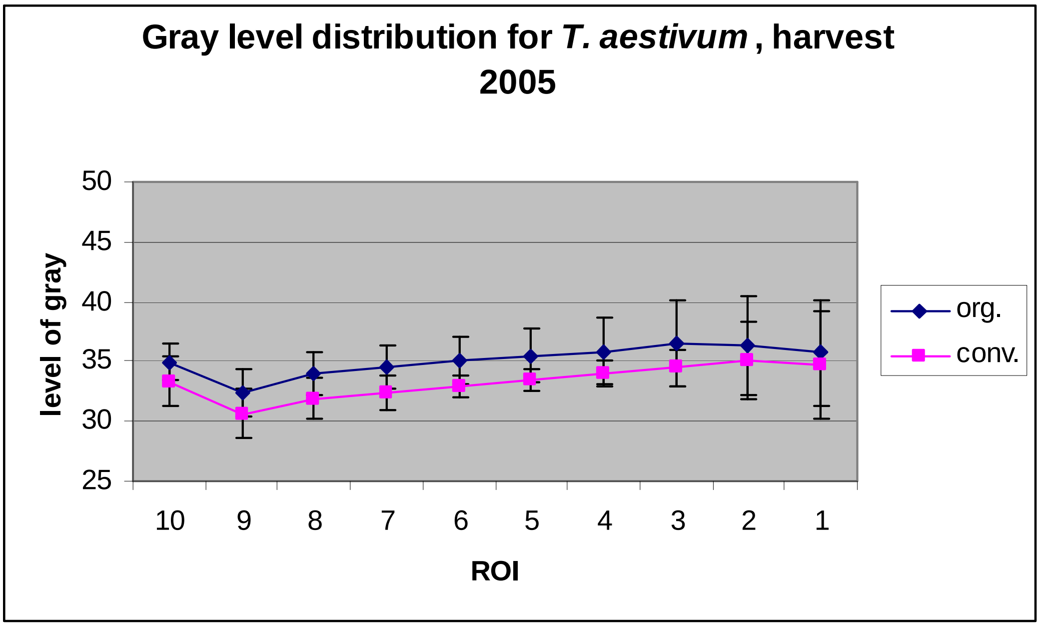
The results showed that the mean values of the grey level distribution for the organically grown samples were higher than for the conventional samples, which means that the patterns were lighter and their crystal layer was denser.

The analysis of the grey level distribution allowed a differentiation between the organic and conventional wheat samples. A significant discrimination was possible for all samples and years for ROI 9, 8 and 7.

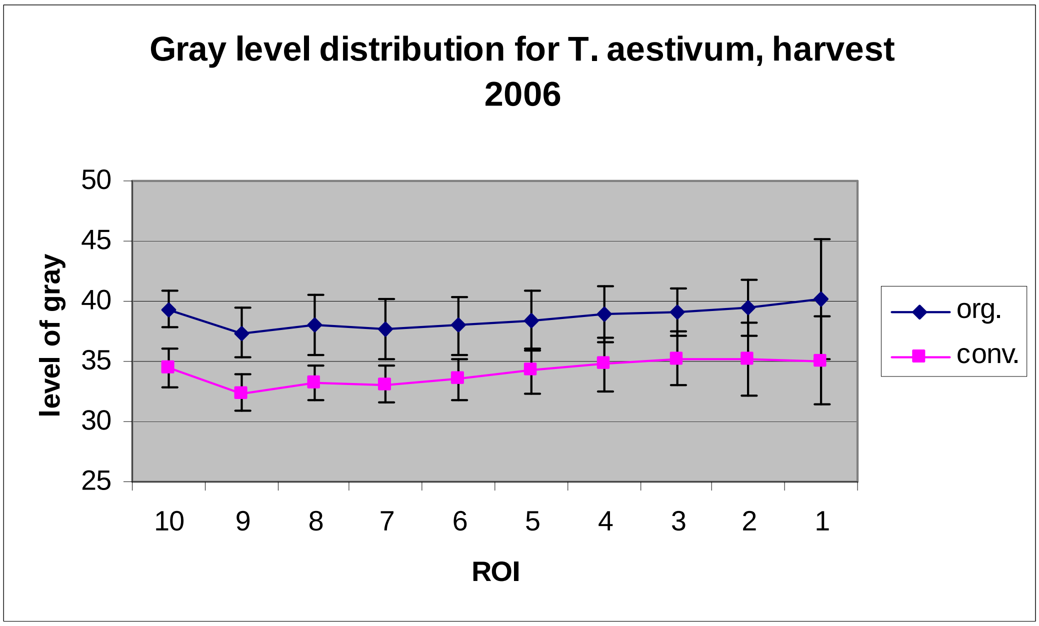
Differences between the two harvesting years

The values of the grey level distribution of the organically and conventionally grown *Triticum aestivum* and *Triticum durum* samples coming from the harvest 2006 were higher than in 2005. It means that the samples harvested in 2006 created during the crystallization a denser crystal structure than the samples harvested in 2005, what confirms the results of the visual evaluation.

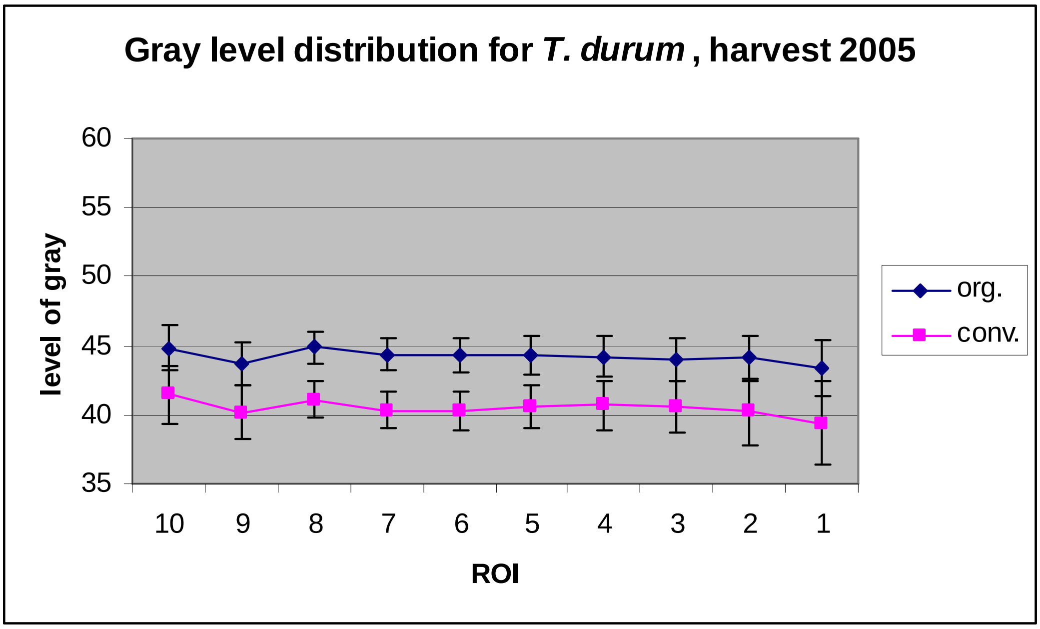
The results are shown in the figures below.



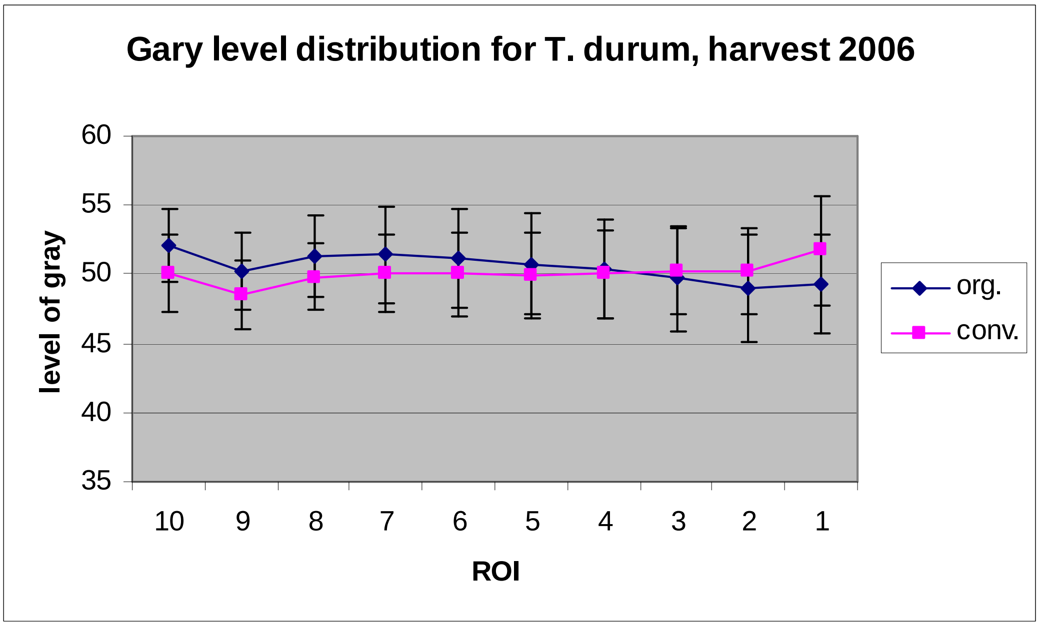
Graphic 12: Grey level distribution



Graphic 13: Grey level distribution



Graphic 14: Grey level distribution



Graphic 15: Grey level distribution

	Significance of the grey level distribution within the ROI's									
	ROI 10	ROI 9	**ROI 8**	**ROI 7**	**ROI 6**	ROI 5	ROI 4	ROI 3	ROI 2	ROI 1
T. aestivum 2005	ns	ns	*	*	*	ns	ns	ns	ns	ns
T. aestivum 2006	***	***	***	***	**	**	*	ns	ns	ns
T. durum 2005	**	***	***	***	***	***	**	**	*	ns
T. durum 2006	**	**	**	*	*	ns	ns	ns	ns	ns

Table 26: Significance of the grey level distribution within the ROI's

The results of the correlation analysis of variables (yield, levels of grey, 1000 corn weight, weight of 1 hl, total protein content, gluten content, and zeaxanthin content) showed that the levels of grey do non correlate with other variables (see table 27). This findings can indicate that the levels of grey do non depend on yield as well as on protein content of the samples and point therefore on another quality dimension, which might be the quality of the product seen as the whole, its coherence, and structural characteristics.

```
Pearson correlation matrix

              YIELD     GREY      CORN     WEIGHTHL   PROT    GLUTEN    ZEAX

 YIELD        1.000
 GREY        -0.145     1.000
 CORN        -0.423     0.427     1.000
 WEIGHTHL     0.425     0.404     0.337     1.000
 PROT         0.764     0.035    -0.547     0.469    1.000
 GLUTPER      0.726    -0.163    -0.238     0.694    0.825     1.000
 ZEAXDM       0.497     0.371    -0.233     0.598    0.783     0.675    1.000
```

Table 27: The pearson correlation matrix for the variables: yield, levels of grey (GREY), 1000 corn weight (CORN), weight of 1 hl (WEIGHT HL), total protein content (PROT), gluten content (GLUTEN), and zeaxanthin content (ZEAX) of the MASCOT wheat samples.

Conclusions

The results of the biocrystallization analysis allowed a differentiation between the two cultivation systems and between the two species. In general common wheat created patterns, which were covered by crystallization needles in a lower degree than the durum wheat patterns. The run of the stems in the common wheat patterns was good visible. Durum wheat patterns had a denser needle structure and the run of the stems was not visible. The organic system seems to produce more regular and well irradiated patterns. Differences in growing conditions in year 2005 and 2006 seemed to have effect on crystallizations patterns, as highlighted by the results. The samples coming from harvest 2006 created during the crystallization denser crystal structures than the samples coming from harvest 2005.

3.5. Experiment IV - Biocrystallization of the wheat gluten from organic and conventional cultivation systems

The experiment was performed at the Biodynamic Research Association Denmark (BRAD) in Hertha in collaboration with Dr. Jens-Otto Andersen.

The goal of the experiment was the discrimination of the organic and conventional *Triticum aestivum* samples on the basis of the gluten pictures. The experiment was preceded with a preliminary study of the gluten biocrystallograms. This preliminary study was necessary for the collection of information about the features appearing in gluten pictures and for the establishment of applied criteria for the visual evaluation.

3.5.1. The preliminary study on the features of the gluten patterns

Background

As resulted in previous studies of Andersen (2006) the gluten pictures are different from the whole wheat pictures and the variation of the gluten pictures is very wide. There are features which are commune for the majority of gluten pictures and the appearance of this features has a varying intensity. The evaluation of features intensity can be successfully used for discriminating the samples.

Materials and methods

The gluten pictures used in the preliminary study derive from the gluten project *"Development and testing of the biocrystallization method for examining gluten quality, with respect to consultancy service on bread-wheat for farmers and processing companies"* (Andersen, 2006). The pictures were examined visually for commune features and the extension in which the features appear. The examination was performed by use of microscope and a light-box of macroscopic features. Photos of pictures have been taken for the documentation of the results.

Results

Macroscopic features

The gluten picture is made of two well defined zones: the middle zone with the centre of the picture, and the outer zone.

The middle zone can be divided into the centre zone and the long radiate zone, but as a huge amount of gluten pictures does not have a well defined centre, this division is not always applicable. The middle zone is filled with stems; the form of the stems can vary from radial (picture 14) through dense radial formations (picture 15) to flexible forms (*beweglich*) (picture 13).

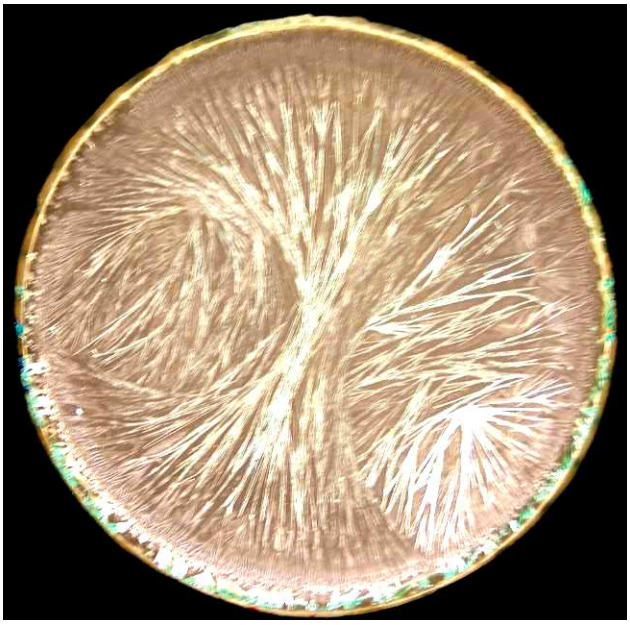
The centre of the picture can be a one-pointed centre (see pictures 13 and 15) or it can be a zoned centre (see picture 16). Lemniscates frequently appear near the centre; their size can vary from little to huge (in many cases the lemniscates don’t fit into the image, as seen on picture 16).

The outer zone can be smoothly connected with the middle zone or delimited from the middle zone by a relatively intensive thinning out (a ring with more or less thin needles or without any structures at all). The outer zone can be filled with crystal structures; and these can create a continuation of the stem structure of the middle zone or form more or less independent, wreath-like structures.

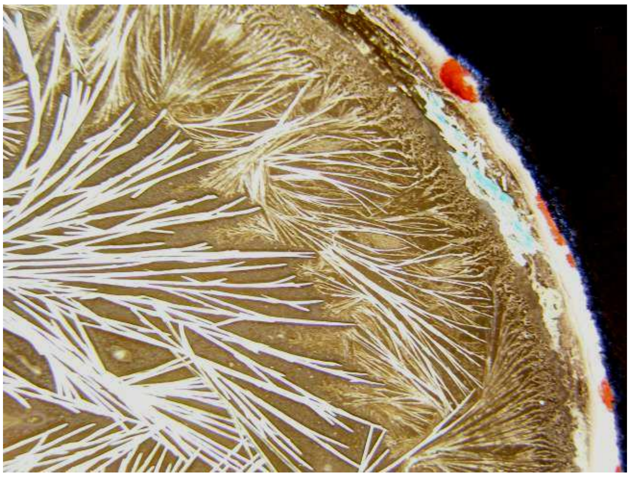
Picture 12: Example of a picture with a well defined centre. The outer zone of the picture is filled with structure. The passage between the zones is smooth; although there is a little thinning out effect. The middle zone is light green

Picture 13: Example of a picture without a centre. The stems are running in wisps (or bundles). The passage between the two zones is smooth; there is no thinning out effect.

Picture 14: Example of a picture with a well defined centre. The middle zone is filed with dense radial formations. Many stems don’t enter into the outer zone, but end abruptly, without ramifications. The thinning out effect between the middle and outer zone is strong. The outer zone is filled with structure, which is partial independent.



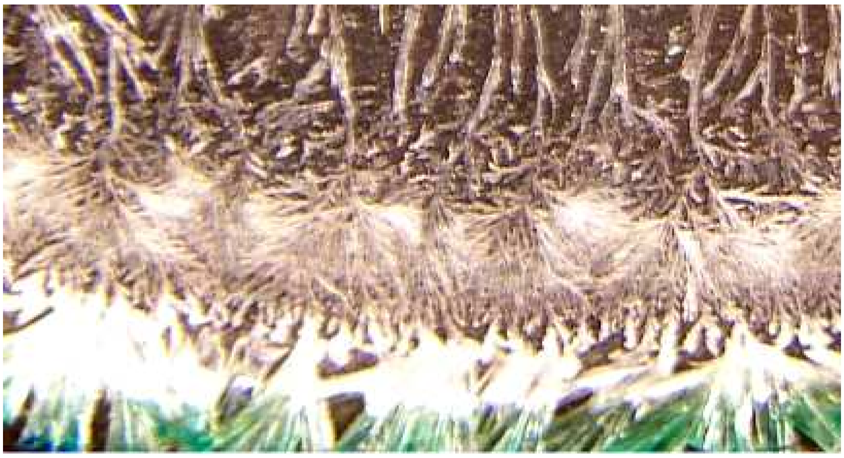
Picture 15 Example of a picture with a zonal centre. The lemniscates are huge and don’t match into the picture. The two zones are well connected; there is no thinning out effect. Inside the outer zone no independent structures appear.



Picture 16: Example of an outer zone with strongly independent structures.

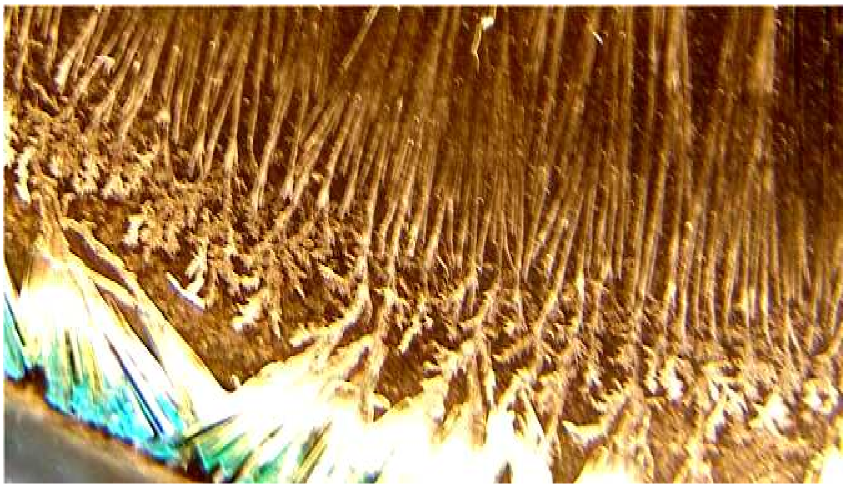
Microscopic features

The peripheral zone of gluten pictures can be more or less developed. The peripheral zone creates a passage between the cupper chloride zone and the outer zone where the ramifications end. More or less bushy dendritic shapes can appear in this zone and can also be connected to the cupper chloride zone and to the ramification zone (see picture 18), or there can be a bare band between the zones without any crystal structures.



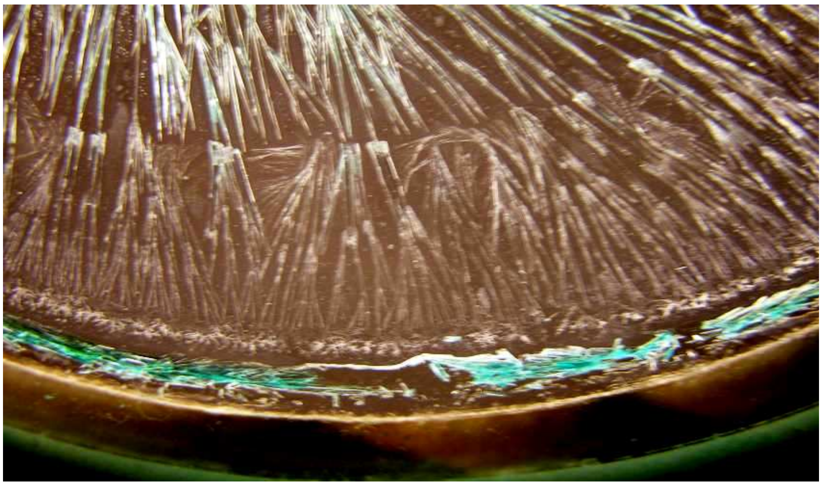
Picture 17: Example of a bushy, well formed peripheral zone with connection to the cupper chloride zone and ramification zone.

Picture 18: Example of a well formed peripheral zone, less bushy than in picture 18 and with a slightly weaker connection to the cupper chloride zone.



Picture 19: Example of a poor peripheral zone. The dendritic shapes are less ramified. There are more free spaces inside the peripheral zone. The connection to the cupper chloride zone becomes weak.

Picture 20: Example of a poor peripheral zone. The dendritic shapes are not well formed. There is no connection to the cupper chloride zone and a weakening of the connection to the ramification zone.

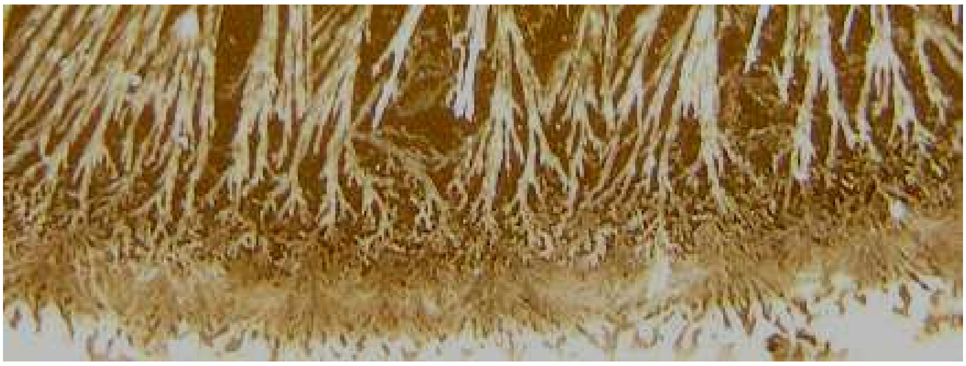


Picture 21: Example of a poor peripheral zone without any connection to the cupper chloride zone, and partial to the ramification zone.

There may be differences in the development of the peripheral zone inside one picture. The peripheral zone of the upper part of a one centred picture is often less developed as the lower part, which develops the most. The degree of development of the peripheral zone located on the left and right side from the centre of the picture can be placed in between the degrees of development of the upper and lower part.

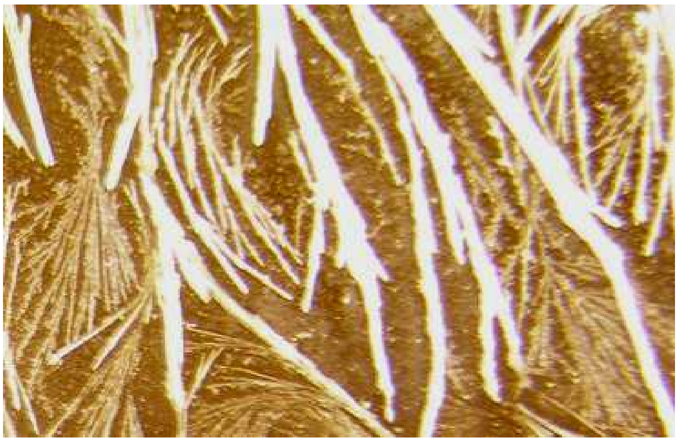
Picture 22: Example of the upper peripheral zone of a one centred picture.

Picture 23: Example of the peripheral zone on the right from the centre of a one centred picture.

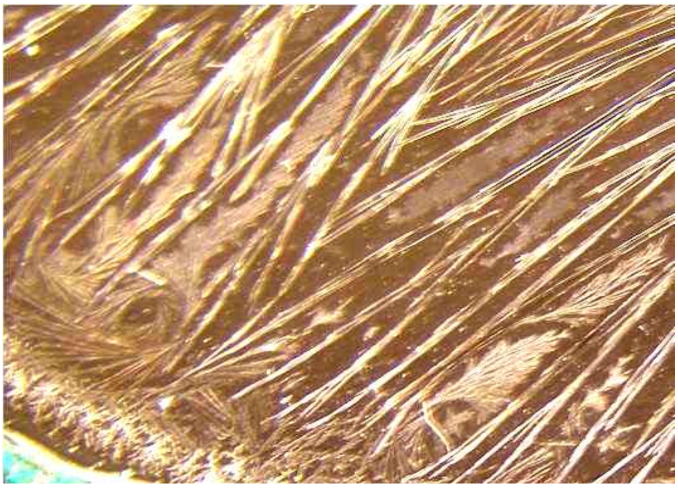


Picture 24: Example of the lower part of the peripheral zone of a one centred picture.

In the ramification zone between the stems, secondary crystallizations can appear. The quantity and structure of these forms can vary. The secondary crystallizations can be visible as well formed, very fine structures, or they can appear as radiate poor structures or spots.



Picture 25: Example of the ramification zone of a picture with well formed, fine shaped and spot-like secondary crystallizations.



Picture 26: Example of the ramification zone of a picture with well formed to feeblish formed secondary crystallizations.

Picture 27 Example of a ramification zone of a picture with well formed, radial secondary crystallizations.

3.5.2. The biocrystallization of the wheat gluten samples

Background

Both baking quality and health proprieties of wheat are strictly correlated with the gluten quality and gluten content.

In accordance with the common opinion, the higher the gluten content the better the baking quality. However experiences from organic bakeries show that organic wheat batches may show good baking proprieties despite low gluten content and gluten index values, especially when alternative fermenting and baking techniques are applied.

A growing percentage of European population shows wheat intolerance and gluten allergy responses to breads and other wheat products produced by means of modern high-yielding varieties. Experiences from nutritional therapists indicate that some persons tolerate better older varieties, specially when these are grown organically and processed with alternative baking techniques.

Thus methods are needed, which may complement traditional methods for assessing baking proprieties, as also methods, which may contribute in clarifying the complex dynamics behind intolerance and allergy responses (Andersen, 2007).

A characteristic feature of the holistic methods is that they work on the whole, undivided product. It could be claimed, that the biocrystallization of gluten isolated out of wheat samples is a denial of this main principle of the holistic methods. Our approach to this question was such that the isolation of the gluten complex is a mechanic process, which can be also performed manually. It does not disturb in any way the products structure or degrade its chemical composition.

According to Hagel (2005) increasing the N content of grain by N-fertilization, of which bakers are very fond of for technological reasons, increases only the S low gluten proteins, not S rich albumins and globulins (Doekes et all., 1982; Wieser et all., 1998), which are important from the nutritional point of view.

The number of people with wheat intolerance is recently growing. Many of them refer a better tolerance to ancient wheat varieties, like spelt, rather then to modern varieties (Hagel, 1999) and to organic wheat products, rather then to conventional wheat products (Andersen, 2007).

Materials and methods

Biocrystallization – The organic and conventional *Triticum aestivum* samples, harvested in 2005 were analyzed at the BRAD laboratory. The samples have been coded and treated as blind samples.

The gluten indexes of all samples have been measured by the use of the Glutomatic system.

The gluten was isolated and used for the preparation of the crystallization solution in accordance to the gluten standard invented by BRAD. The crystallization of the gluten samples was performed in a two day repetition; each time six picture replicates of each sample had been prepared (36 plates plus 7 standard gluten pictures).

During the 15th hour of the crystallization the pictures were checked visually for determining the crystallization time. Each time the degree in which the plate was already covered by crystals was measured.

Visual evaluation - For the visual evaluation four pictures of each sample have been chosen. The put away pictures were chosen in accordance to following criteria in the following sequence: technical error, time effect, multi centred, overall impression, randomised selection.

The four pictures of each sample have been evaluated visually by three evaluators. The evaluation was performed as a quantitative ranking, based on a discrete scale of 1-12, with units of 1, assumed equidistant. The reference pictures with units 1,4,7,9 and 12 were selected in agreement among 3 evaluators from the overall amount of pictures in the project, and thus are internal. The samples were blind to the three evaluators for all practical purposes. The samples were evaluated visually on the light box (4 pictures per each of the 6 samples were placed on the light box simultaneously).

For the evaluation of the microscopic criteria (peripheral zone) two pictures per sample have been chosen and the evaluation area has been specified. The two pictures have been chosen in accordance to the following criteria: one centred picture, randomised choice. In the one centred pictures an area lying on the left or right side from the centre was selected (the selection of the upper and lower part of the peripheral zone has been avoided, because of the differences in the development of this zone in this areas). Also an area of evaluation has been specified on the reference pictures, to avouch that all evaluators have exactly the same conditions for the evaluation.

The samples have been evaluated according to the following criteria:

Macroscopic criteria:

Centre coordination: The degree to which a centre is present from where the form elements are coordinated in a polarity between an absence of a centre (1) and a distinct centre (12), from where radial and ramification structures cover the whole picture.

Integration: The level to which the single form elements constitute on overall structure in a polarity between a random distribution of form elements over the plate (1) and an experienced unified, whole picture (12).

Irradiation – static approach: The extent to which the radial or ramification structure of the middle zone is expanding towards the periphery in a polarity between a structure observed only in the centre and middle zone (1) and a structure which not interrupted is expanding from the centre zone to the periphery zone (12).

Mobility/flexibility: The point to which an organic movement of the form elements can be experienced in a polarity between a “stiff”, radial formation of long needles (1) and a curved structure with regular zone transitions (12).

Amorphous and polygonal formations in the middle zone: The extent to which isolated amorphous and polygonal formations in the middle zone are observed. The formations are not integrated into the radial or ramification structure, and which are surrounded by a crystal free zone, in a polarity by numerous such formations (1), and none (12).

Felting: The degree to which a felt-like or irregular interwoven formation is observed in the middle zone. It is delimited “inwardly” by 90° angle around the vertical axis, and outwardly by a peripheral distance of 15 mm, in a polarity between the absence of such a formation (1), and the presence of a fully interwoven formation (12).

Microscopic criteria:

Dissolution of the peripheral zone: The level to which the outer zone (the combined $CuCl_2$ zone and transition zone) is dissolved in a polarity between an absence of crystals (1), through the presence of numerous spots and streaks between the transition zone and the CuCl2 zone, and a fully developed ramification structure connected to the stem structure of the middle zone (12).

Results

The results showed, that four of the criteria applied in the visual evaluation of the gluten pictures allowed a successful sample discrimination between the two cropping systems. The discriminating criteria were the following ones: centre coordination, integration, amorphous and polygonal formations, dissolution of the peripheral zone.

The table below shows the degree (in scale from 1 to 12) in which the feature appears in the pictures.

	Centre coordination	Integration	Amorphous and polygonal formations	Dissolution of the peripheral zone
T. aestivum				
conventional	**6,8**	**5,8**	**8,3**	**6,1**
organic	**9,4**	**9,0**	**9,7**	**8,3**
P values for treatment	<0,0001	<0,0001	<0,0001	0,003

Table 28: Intensity of the appearance of features (in scale from 1 to 12) in the gluten pictures crystallized out of conventional and organic Tritium aestivum samples.

Organic samples seem to produce images with higher centre coordination and irradiation. More over the pictures tend to have less amorphous formations present in the middle zone. The connection between the middle and the peripheral zone is continuous, without a thinning out effect (see values for the dissolution of the peripheral zone). In general organic samples seem to create better developed and balanced pictures.

The gluten index determined on the same samples did not reveal such significant differences between the two cropping systems (average gluten index in organic wheat was 83.6 and in conventional 80), therefore the differences in the morphological features of the pictures could be related to the quality of the gluten complex in a more complex manner.

Conclusions

The biocrystallization method of gluten allowed a significant differentiation of the blinded organic and conventional *Triticum aestivum* samples. This shows that the method is very sensitive to factors influencing the protein metabolism like the N-fertilisation.

The experiment, as part of the gluten project, has generated unique knowledge about the ability of the biocrystallization method to discriminate wheat samples, not only on the basis of the whole-grain samples which is well known from earlier investigations, but from gluten as a single protein fraction.

The results gained in the biocrystallization analysis of gluten showed, that the gluten isolated out of organic and conventional samples differed markedly in the picto-morphological proprieties. It might be, that the cultivation system affects not only the gluten quantity in grain but also its structural features. To our knowledge there is until now no study on the tolerance of organic and conventional wheat by humans. The biocrystallization method could result as an appropriate and useful method in such investigations.

The project results encourage further studies in relation to a possible link between the picture-developing proprieties of gluten samples and intolerance/allergy responses in humans. It is noteworthy that organic samples show better picture-developing proprieties than conventional samples. It is probable that organic wheat has a better balanced N-metabolism (less nitrate, more pure protein, higher EEA-index) than the conventional one. Therefore the method may contribute in a better understanding of the complex correlations between N-fertilization and protein metabolism of the wheat crop and on the other side wheat intolerance and gluten allergy responses in humans (Andersen, 2007).

3.6. Experiment V - Degradation test

Background

The ability of a crop to reach physiological ripeness at harvest is an important quality parameter in the organic quality research, as well as the ability to preserve crop-specific characteristics (outer morphological characteristics, colour, taste, aroma, content of nutritional compounds) during post-harvest conditions. Correspondingly there is an interest in degradation tests, i.e. methods for determining the ability of crop and food samples to preserve these characteristics during stressful conditions (Andersen, 2005).

Therefore biocrystallization could represent an useful method to investigate the quality of agricultural products by means of degradation tests. A plant extract is produced on the basis of e.g. a wheat sample, and stored in a refrigerator at 3℃, thereby exposed to a non-specific autolytic and microbial degradation. When producing biocrystallization patterns over a number of successive days, systematic progressive changes in morphological features are observed, specifically a dissolution of the original co-ordinated structure. This is a parallel to the chemical degradation of macromolecules, such as proteins, into smaller molecules which can be followed analytically. The speed at which this dissolution of the forms viewed in the patterns takes place is regarded as a qualitative parameter, analogous to storage ability (Engqvist, 1970).

Materials and methods

Extracts of conventionally and organically grown samples of the two examined species: *Triticum durum* and *Triticum aestivum* were allowed to older in controlled conditions (the extracts were stored in a refrigerator at 3ºC). Biocrystallograms were generated at the 1st, 3rd, 5th and 8th day of the degradation period.

The obtained patterns were scanned and analysed by the use of the computer software *Image J* for the grey level distribution.

The features of the crystal patterns were analysed visually.

Results

During the deterioration process there is a growth of the quantity of microorganisms in the extracts and a progressive degradation of the big molecules, like proteins, into smaller ones. The growth of the microorganisms mass most probably causes the growth of structures density in the pictures, whereas the degradation of molecules inducts the appearance of chaotic, irregular forms.

The higher density of created forms in the pictures made out of older extracts and the growing degradation of forms could be seen in the patterns during the visual evaluation and confirmed in the computer supported evaluation by use of the software *Image J*.

The visual evaluation

The visual evaluation of the patterns (see appendix 3) showed that the differences between the organically and conventionally grown common wheat samples (see chapter 9.4.) are still visible in the pictures prepared out of 3 days old extracts, however in older extracts (after the 5th day of degradation) the differences between the organic and conventional variant are lost.

Also the differences between the two wheat species, *Triticum aestivum* and *Triticum durum* (see chapter 9.4.), get less visible with the progress of the degradation process.

The differences between the field replicates seems on the other hand to be more constant during degradation and therefore visible even in pictures prepared out of 8 days old extracts. It seems that in some cases the differences between the three field replicates get even more visible with the progress of the degradation process. This might be caused for instance by different microbial contamination of the wheat kernels coming from different field replicates. This issue needs further investigation.

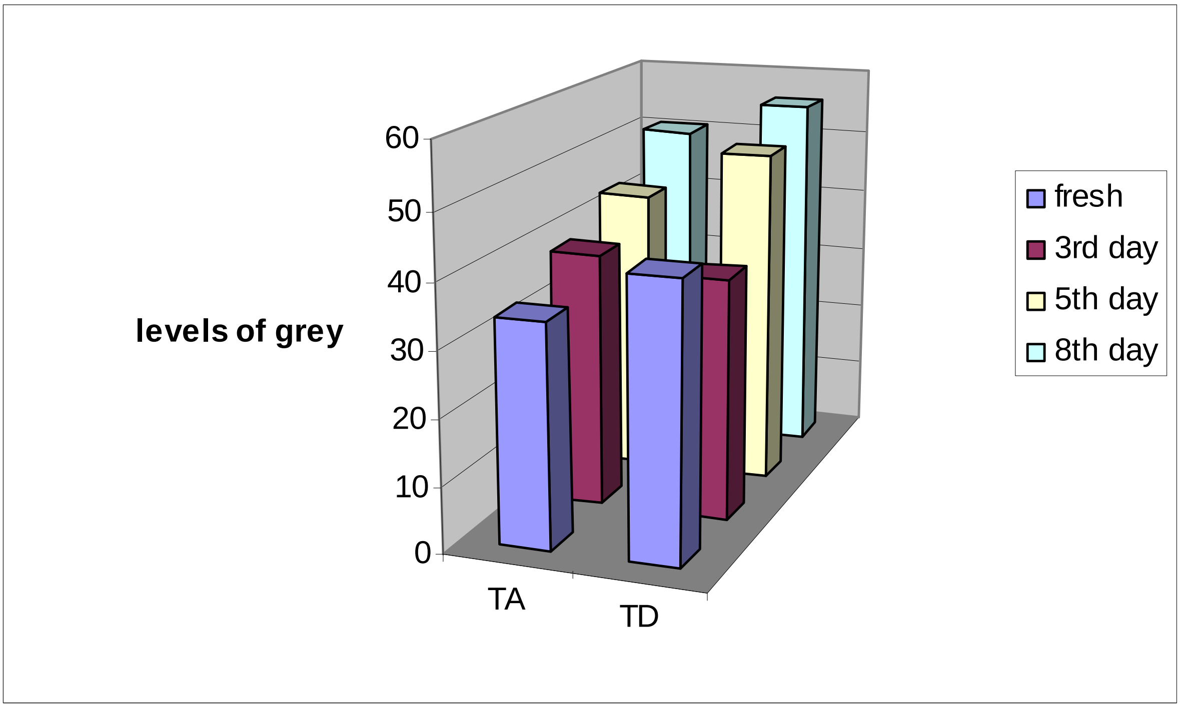
With the progress of the degradation process of the extracts the resulting pictures showed a progressive growth of irregular forms, made out of short needles, with many oblique needles (*Quernadeln)* and dense radial formations. The smooth forms, which appear in pictures made out of fresh extracts and other typical features, which regarded the sample quality, get lost.

Picture 28: Needle structures of the middle zone of biocrystallization patterns produced out of a Triticum aestivum extracts: (from the left) fresh, 5 days old, 8 days old.

Picture 29: Needle structures of the middle zone of biocrystallization patterns produced out of a Triticum durum extracts: (from the left) fresh, 5 days old, 8 days old.

Analysis of the grey level distribution

The degradation period of the analysed extracts had an influence on the grey level distribution values of the resulting pictures. The longer the degradation period, the lighter the resulting pictures, and the higher the values of the grey level distribution. In the graphic below the mean values of the grey level distribution for the fresh, 3 days old, 5 days old and 8 days old *Triticum aestivum* and *Triticum durum* extracts are shown:



Graphic 16: Grey level distribution of fresh, three, five, and eight days old *Triticum aestivum* (TA) and *Triticum durum* (TD) extracts.

The higher values of the grey level distribution of the pictures made out of older extracts in comparison to the pictures made out of fresher extracts indicate the growth of the density of crystal forms in the biocrystallization pictures.

The visual analysis results suggest that the differences between the two systems are significant: the grey levels values were 5 and 8% higher in the organic system for common and durum wheat respectively. The difference between the two systems disappeared after the 5th day of extract degradation for the common wheat samples, confirming the visual data results. For durum wheat the grey level distribution values for the pictures made out of three days old extracts were lower than for the fresh ones. The differentiation between the two cropping systems was significant with higher grey level values for the organic variant. The grey level values increased in the fifth and eight day of degradation. In the fifth day the organic variant showed lower grey level values than the conventional one, i.e. the opposite in confront to the results from other days. In the eight day there were no significant differences between the two cropping systems for both analyzed species.

	Triticum aestivum			
	fresh extracts	3 days old extracts	5 days old extracts	8 days old extracts
organic	**34.96**	**40.02**	**43.55**	**53.62**
conventional	**33.36**	**38.03**	**43.94**	**53.46**
significance	p=0.0062 **	p=0.0118 *	p=0.4542 **ns**	p=0.8614 **ns**

Table 29: Grey level distribution values of fresh, three, five and eight days old organic and conventional *Triticum aestivum* samples.

	Triticum durum			
	fresh extracts	3 days old extracts	5 days old extracts	8 days old extracts
organic	**44.20**	**39.71**	**50.76**	**55.98**
conventional	**40.49**	**34.50**	**52.54**	**55.54**
significance	p=0.0000 ***	p=0.0000 ***	p=0.000 ***	p=0.3303 **ns**

Table 30: Grey level distribution values of fresh, three, five and eight days old organic and conventional *Triticum durum* samples.

Conclusions

The results of the degradation test showed a clear differentiation of forms appearing in the crystal patterns with the progress of the degradation period. The differences between the two cultivation systems were visible until the 3rd day of degradation, whereas the differences between the field replicates remained visible until the 8th day and in some cases seemed to become more evident. Then again the differences between the two species got less visible with the progressing degradation. Considering the results of this study, the crystallization method might be applied in analyzing the freshness of a product. This ability could be particularly useful for fresh eaten foods, like fruits and vegetables.

4. CONCLUSIONS

The results showed, that the two wheat varieties: *Triticum aestivum* and *Triticum durum*, coming from the MASCOT experiment differed markedly depending on the cultivation system (organic and conventional). It has been found that the cultivation system significantly influenced the following features of the analyzed species: yield, protein content, technological parameter of the dough, sensorial features of bread, and picto-morphological proprieties of flour and isolated gluten during the biocrystallization analysis. Thus it can be claimed that the cultivation system influenced the qualities of the two wheat species in question.

	Organic	Conventional	Org. scores…	Significance
Yield (/ha)				
TA	3.1	4.9	-	**
TD	3.5	4.8	-	*
Protein content (%)				
TA	10.2	12.9	-	*
TD	9.7	11.8	-	**
Weight of 1 hl (kg)				
TA	80.3	82.1	=	Ns
TD	80.1	82.1	=	Ns
SDS (ml)				
TA	41.4	55.9	-	*
TD	30.4	41.6	-	*
Gluten content (%)				
TD	7.0	8.9	-	*
Gluten Index				
TD	90.5	87.6	=	Ns
W (J 10^{-4})				
TA	141.0	215.0	-	**
TD	117.0	103.0	=	Ns

	Organic	Conventional	Org. scores…	Significance
P/L				
TA	1.3	0.7	-	*
TD	1.4	0.9	=	Ns
Ashes content (%)				
TD	1.8	1.9	-	*
Total phenolic content (mg gallic acid equivalents g $^{-1}$)				
TA	0.46	0.46	=	Ns
TD	0.38	0.35	=	Ns
Lutein content (µg g $^{-1}$)				
TA	1.10	1.00	=	Ns
TD	3.05	3.37	=	Ns
Zeaxanthin content (µg g $^{-1}$)				
TA	0.22	0.22	=	Ns
TD	0.24	0.29	-	*
Preference test (*Which bread do You prefer?*)				
TA	59%	32%	+	***
Grey level distribution values				
TA	36.0	32.5	+	**
TD	48.1	45.5	+	**
Crystallization of the gluten samples (TA)				
Centre coord.	9.4	6.8	+	***
Integr.	9.0	5.8	+	***
AP form.	9.7	8.3	+	***
Diss. P zone	8.3	6.1	+	**

Table 31: Comparison of the results of common and holistic methods of analysis for organic and conventional *Triticum aestivum* (TA) and *Triticum durum* (TD) samples; (+) organic samples score better than conventional samples, (=) no difference, (-) organic samples score less well than conventional samples, visual criteria for the evaluation of gluten patterns: (Centre coord.) Centre coordination, (Integr.) Integration, (AP form) amorphous and polygonal formations, (Diss. P zone) dissolution of the peripheral zone.

The **grain yield** was always lower in the organically managed system. The grain yield of the durum wheat was lower by 18% in 2005, by 23% in 2006 and by 57% in

2007 whereas the yield of the common wheat was lower by 54% in 2005, by 19% in 2006 and by 41% in 2007.

The **protein content** of the organically grown grains was 18-20% lower then in conventionally grown grains. The two species showed approximately the same protein content (11 and 12% in average for durum wheat and common wheat) and in both species the protein content increased, also in the organic variants, from 2005 to 2007.

The organic and conventional grains and flours differed significantly in their **technological parameters**. Because of the better results in the analysis of dough prepared out of conventional flour by use of the Alveograph it can be assumed that the conventional grown grains have better bread making proprieties. Additionally the conventional samples showed higher gluten values in confront with the organic samples. The higher elasticity of dough and higher gluten content, as also the better results in the SDS test make the conventional flour more suitable for bread making.

Although the conventional samples showed higher protein contents, gluten contents and better technological parameter of dough, in the **preference test** of organic and conventional bread, the organic bread was preferred by the majority of the interviewed people (61% of women and 57% of men). It can be claimed that conventional flour produced “soft” bread while organic flour produced bread that was judged as slightly hard and more flavourful.

The results of the **biocrystallization analysis** allowed a significant differentiation between the two cultivation systems. The patterns prepared out of organically grown samples created denser crystal structures during the crystallization in comparison to the conventional samples. In the grey level analysis the organic samples showed higher grey level values than the conventional samples, which means that the organic patterns were created out of denser structures. There were also differences in some of the visually evaluated features. The organic samples created patterns with more substance spirals, more side needles and a slightly stronger *Durchstrahlung* (perradiation), while the patterns of the conventional samples showed more *Quernadeln* and dense radial formations.

Generally the patterns of both species and cultivation systems coming from harvest 2006 showed denser structures and stronger forms than the patterns created out of samples harvested in 2005.
The biocrystallization analysis of the gluten isolated out of *Triticum aestivum* samples harvested in 2005 allowed also a significant differentiation of the organic and conventional samples. The results showed, that four of the criteria applied in the visual evaluation of the gluten pictures allowed a successful sample discrimination between the two cultivation systems. The organic samples created during the crystallization were markedly stronger and had better integrated forms than the conventional samples.

It can be claimed that the lower protein and gluten content and weaker technological parameter of dough of the organic wheat samples did not negatively affected the quality of the product. The preference test showed that the organic bread was preferred by the panellists and the biocrystallization analysis showed better picot-morphological proprieties of the organic whole flour and gluten. The lower technological value of organic wheat showed by means of common analysis could not be confirmed in the sensoric test and holistic analysis, which might indicate, that although the lower protein and gluten content and a weaker technological parameter of dough, the organic wheat had a better quality than the conventional wheat.

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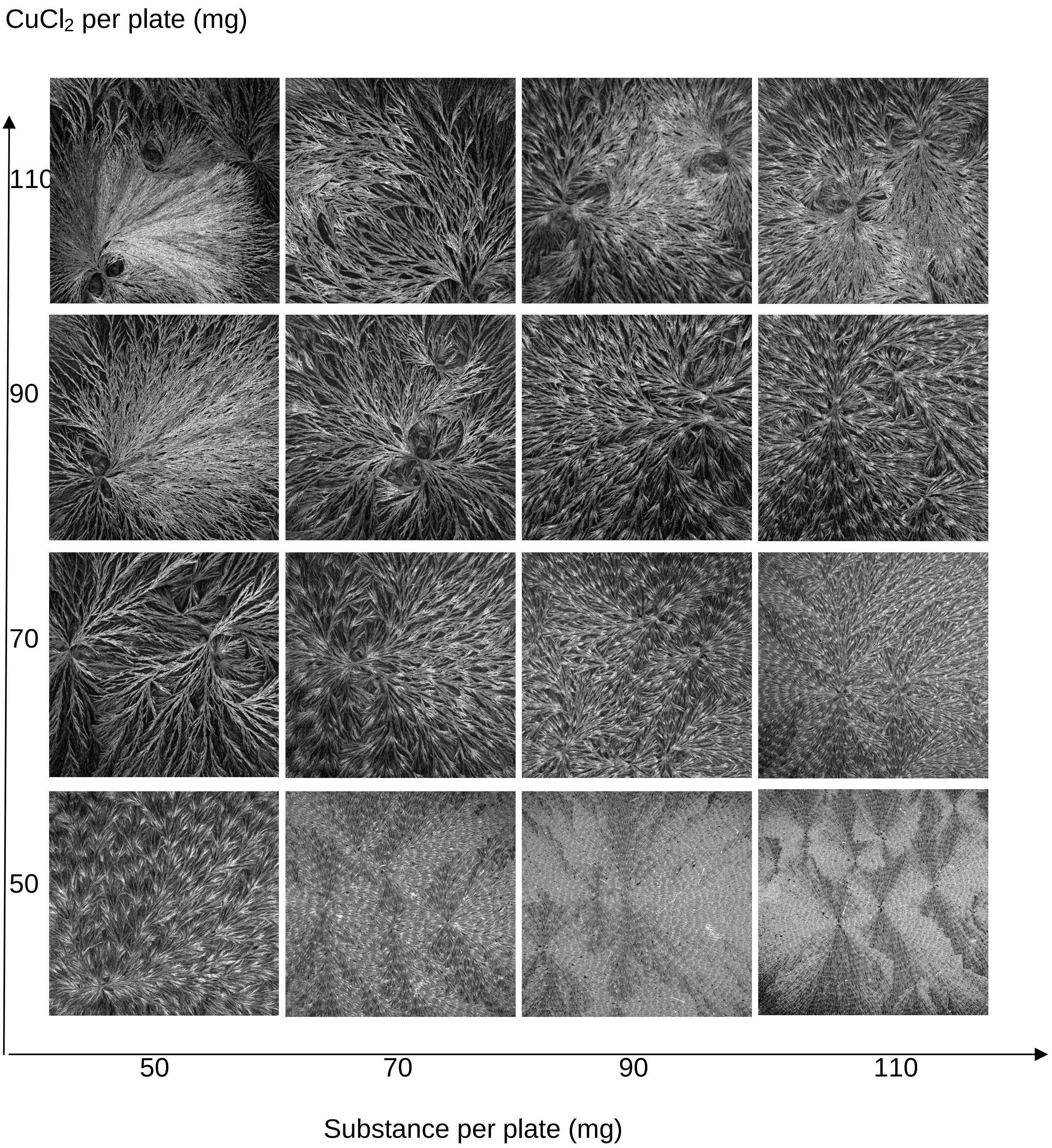
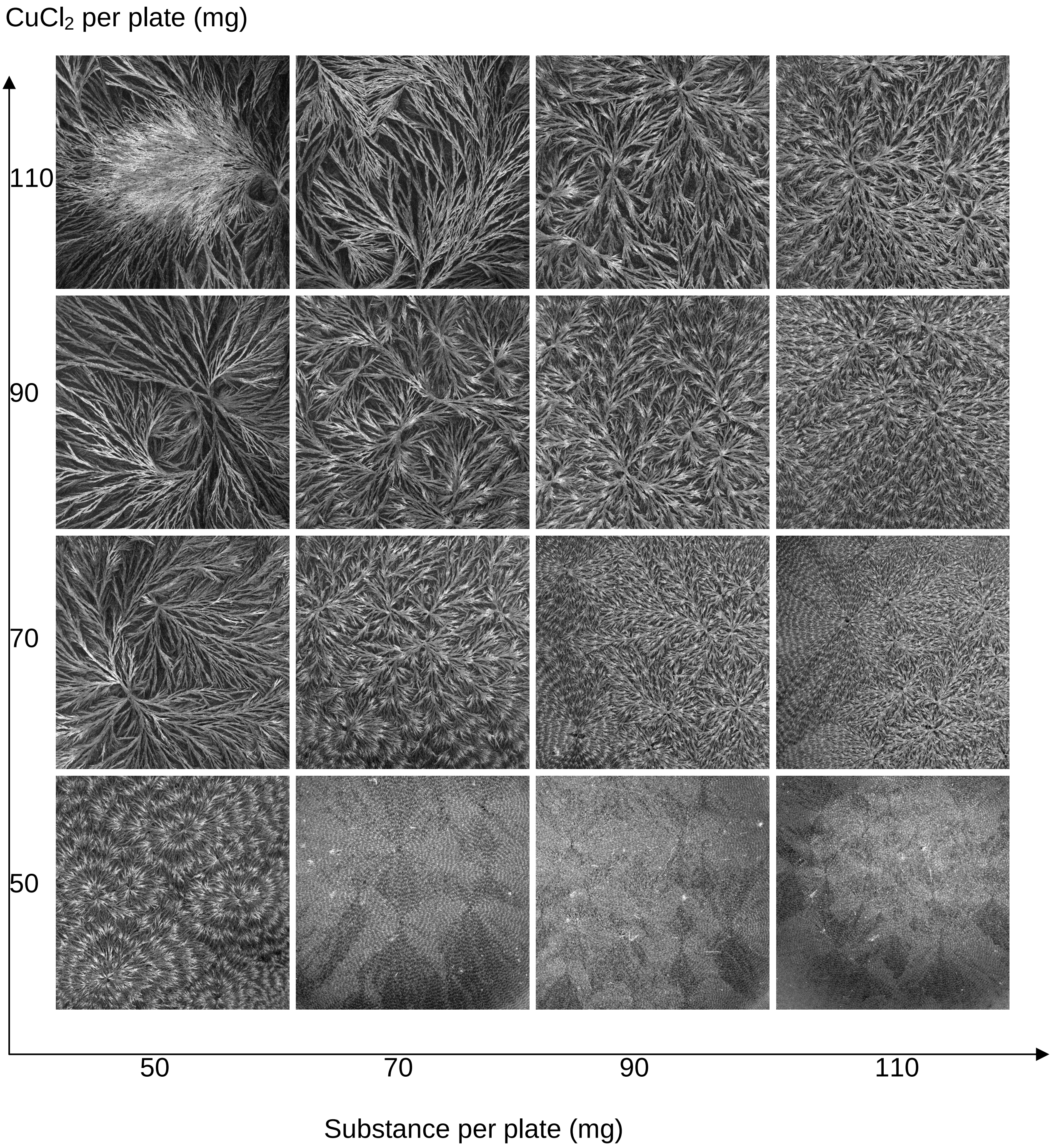
6. APPENDIX 1 – Concentration matrixes

Concentration matrix for *Triticum durum*



Concentration matrix for *Triticum aestivum*



7. APPENDIX 2 – Biocrystallization patterns (whole flour)

Sample no. 1

Sample no. 11

Sample no. 26

Patterns of organically grown *Triticum durum* samples, harvest 2005

Sample no. 6

Sample no. 23

Sample no. 36

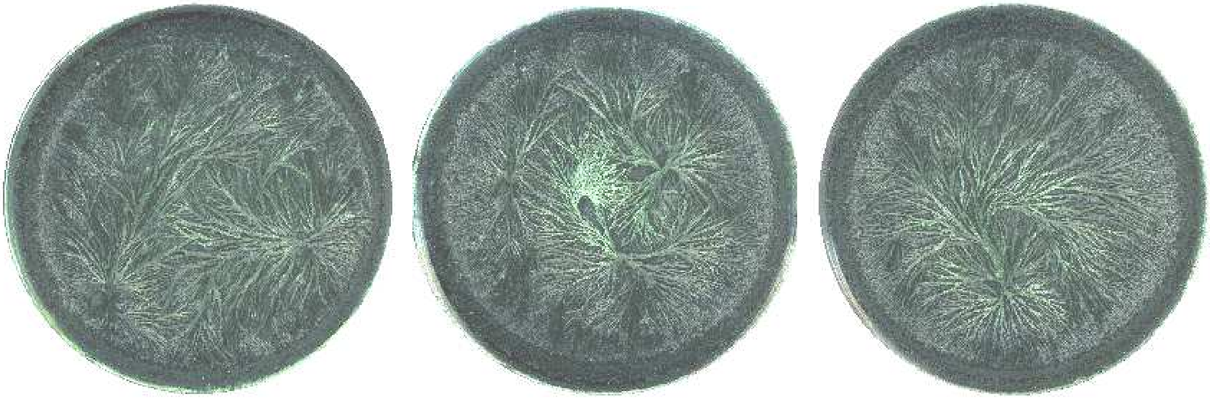
Patterns of conventionally grown *Triticum durum* samples, harvest 2005

Sample no. 3

Sample no. 13

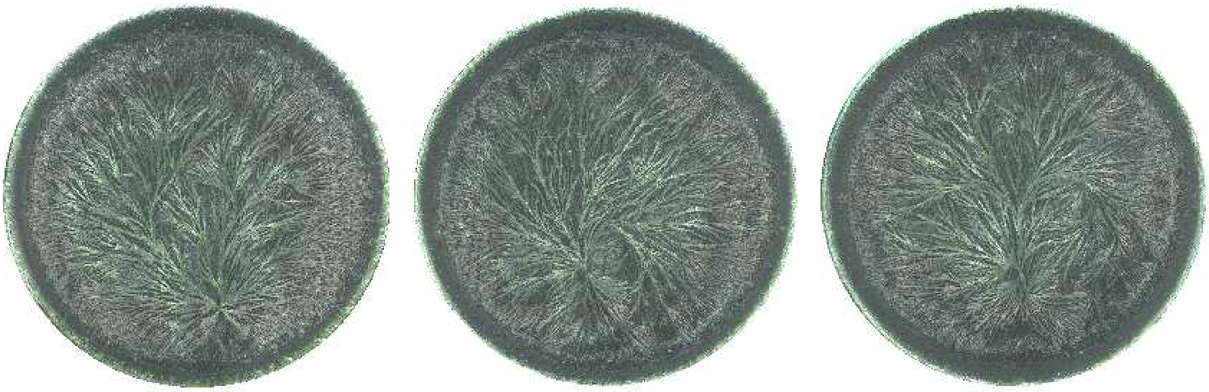
Sample no. 28

Patterns of organically grown *Triticum aestivum* samples, harvest 2005



Sample no. 8

Sample no. 21



Sample no. 38

Patterns of conventionally grown *Triticum aestivum* samples, harvest 2005

Sample no. 5

Sample no. 15

Sample no. 30

Patterns of organically grown *Triticum durum* samples, harvest 2006

Sample no. 10

Sample no. 25

Sample no. 40

Patterns of conventionally grown *Triticum durum* samples, harvest 2006

Sample no. 2

Sample no. 12

Sample no. 27

Patterns of organically grown *Triticum aestivum* samples, harvest 2006

Sample no. 7

Sample no. 22

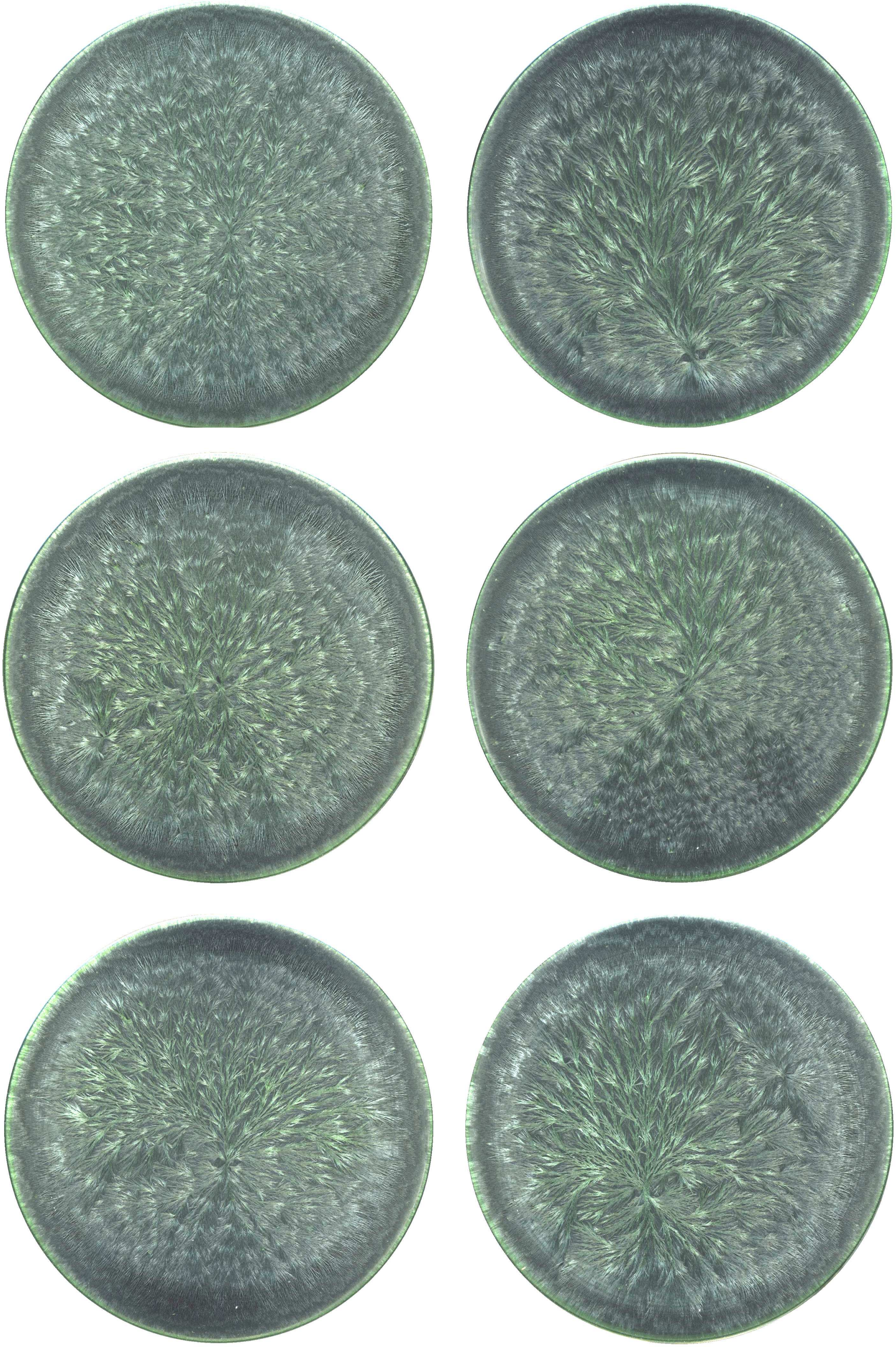
Sample no. 37

Patterns of conventionally grown *Triticum aestivum* samples, harvest 2006

8. APPENDIX 3 – Biocrystallization patterns (degradation test)

Triticum durum, fresh extracts

Organic **Conventional**

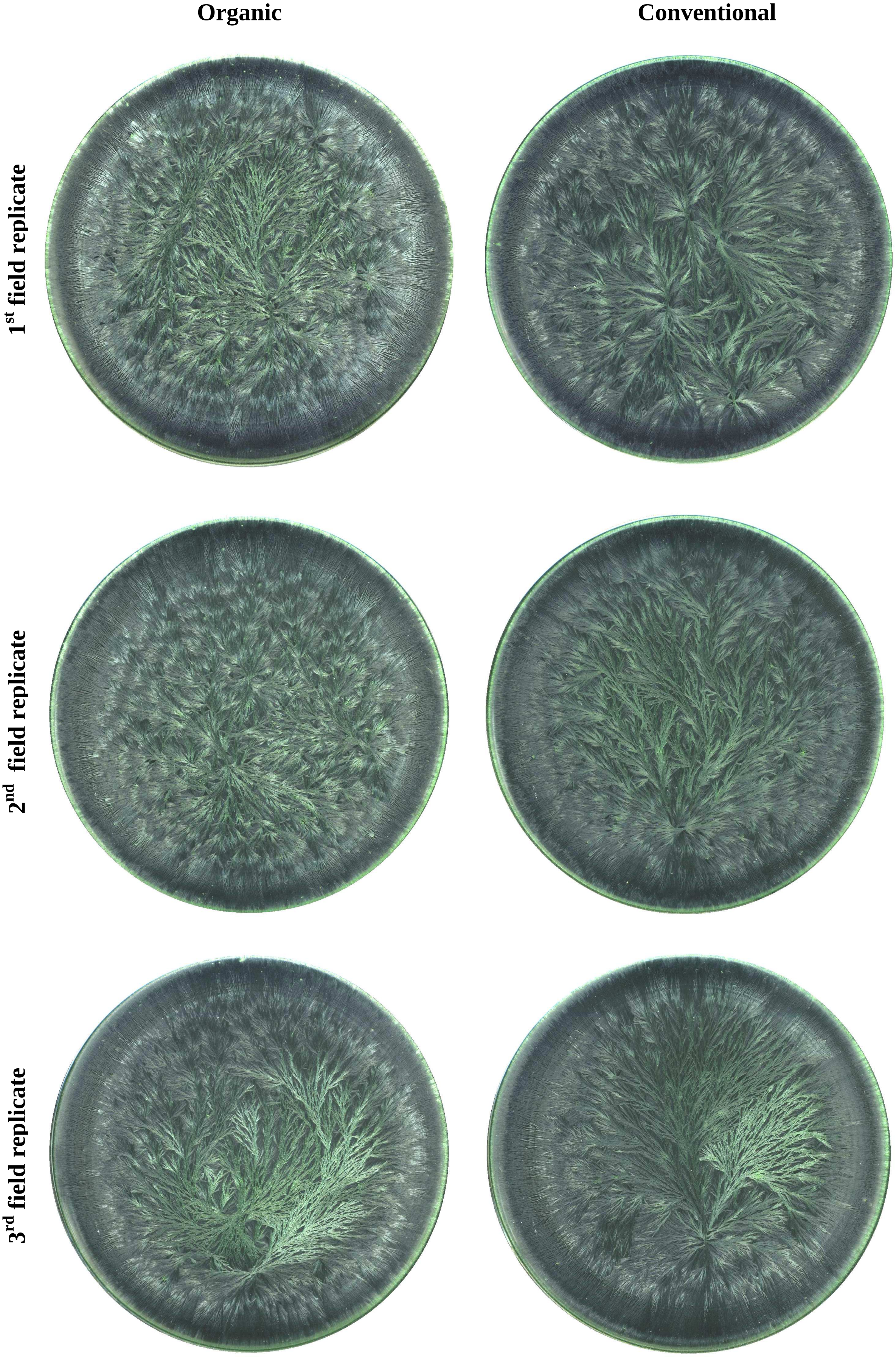


1st field replicate

2nd field replicate

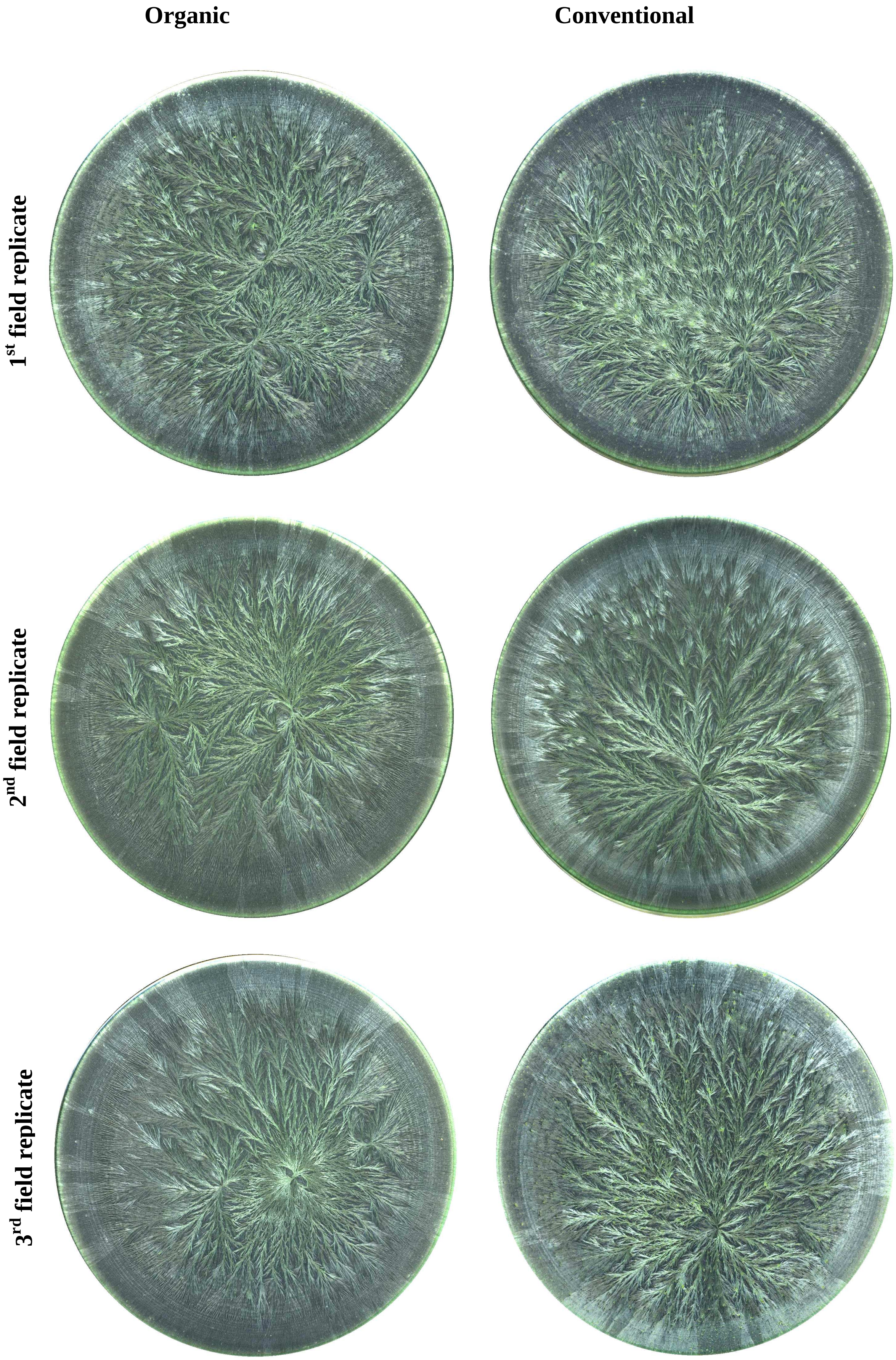
3rd field replicate

3 days old extracts

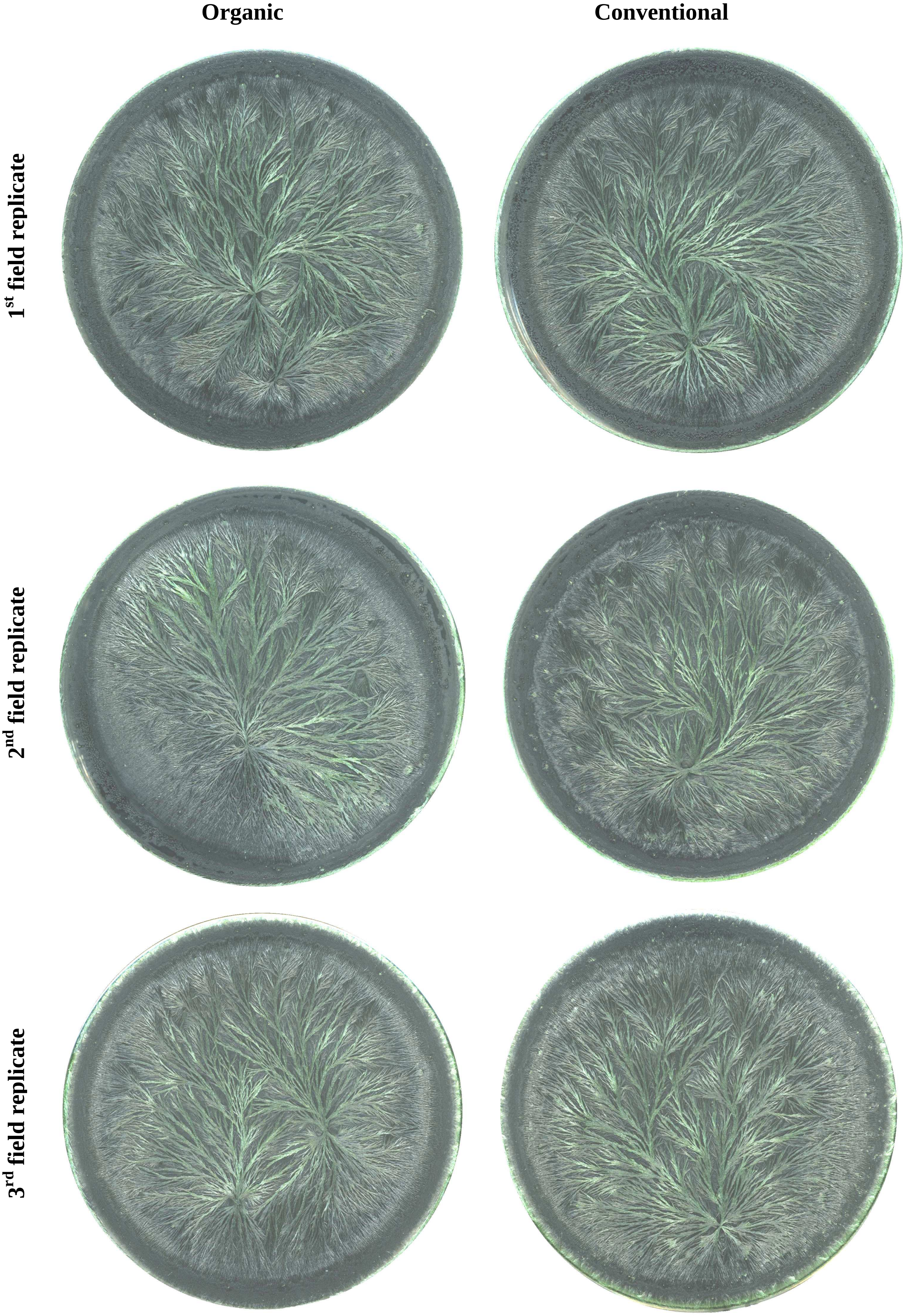


5 days old extracts

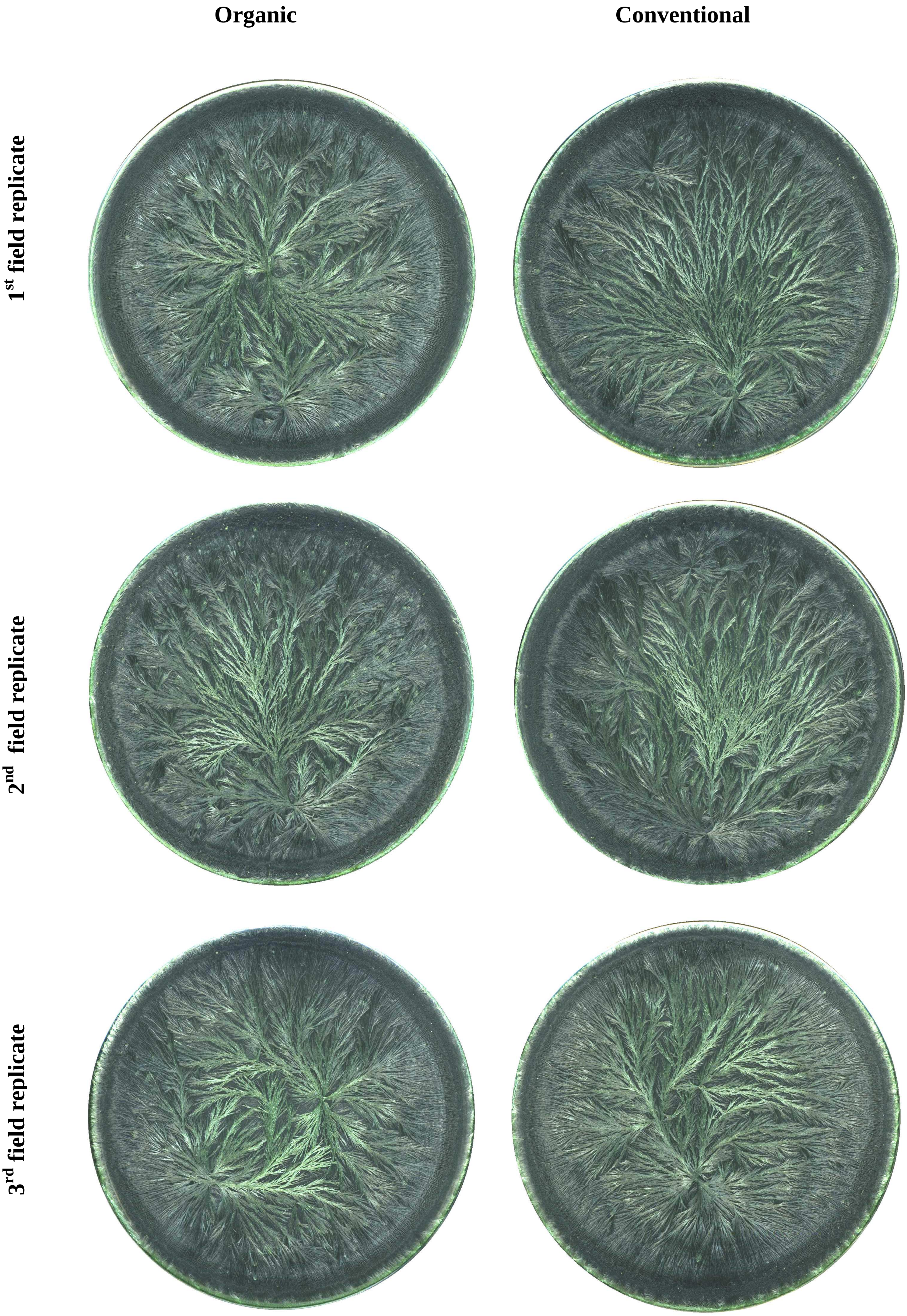
8 days old extracts



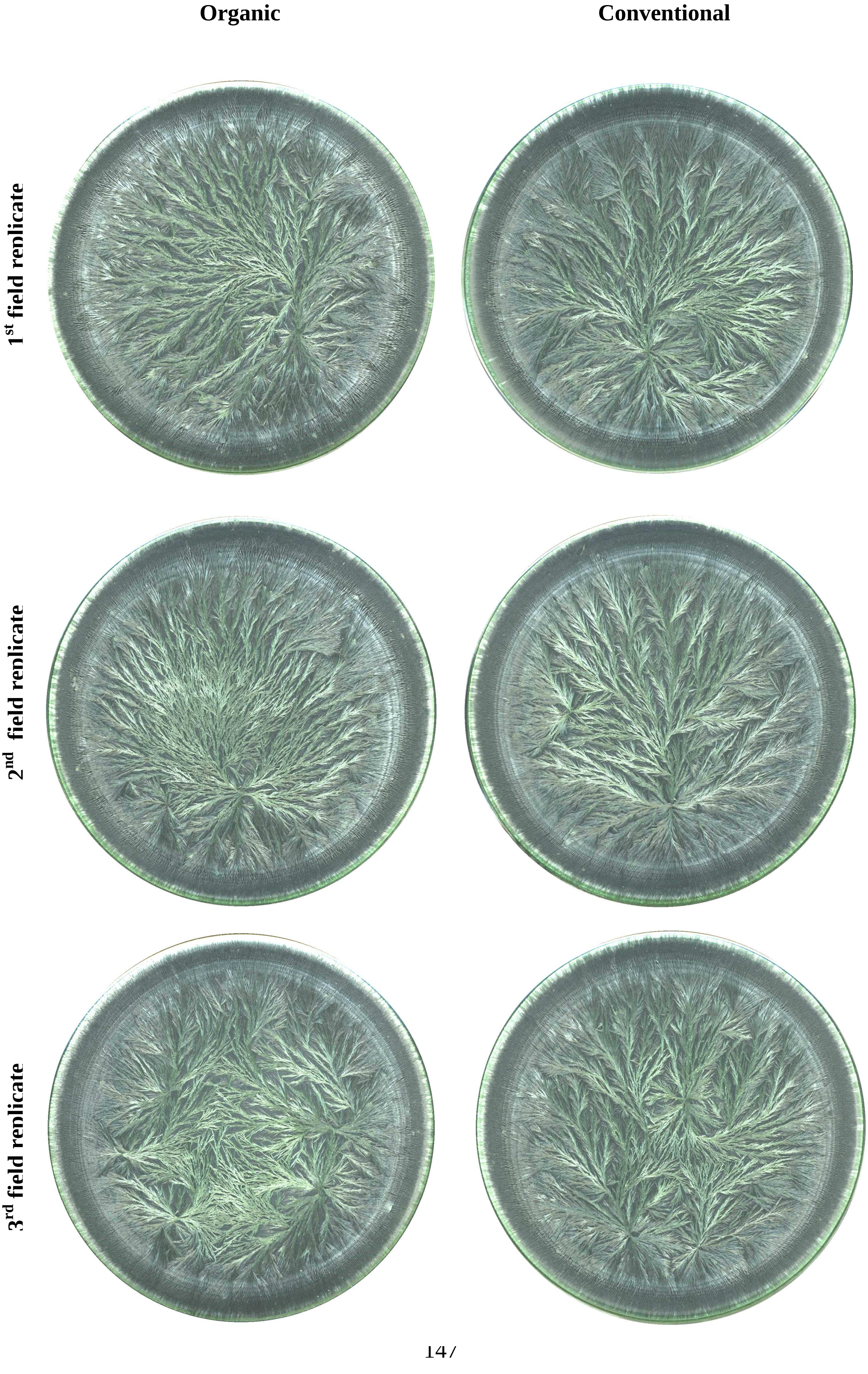
Triticum aestivum, fresh extracts



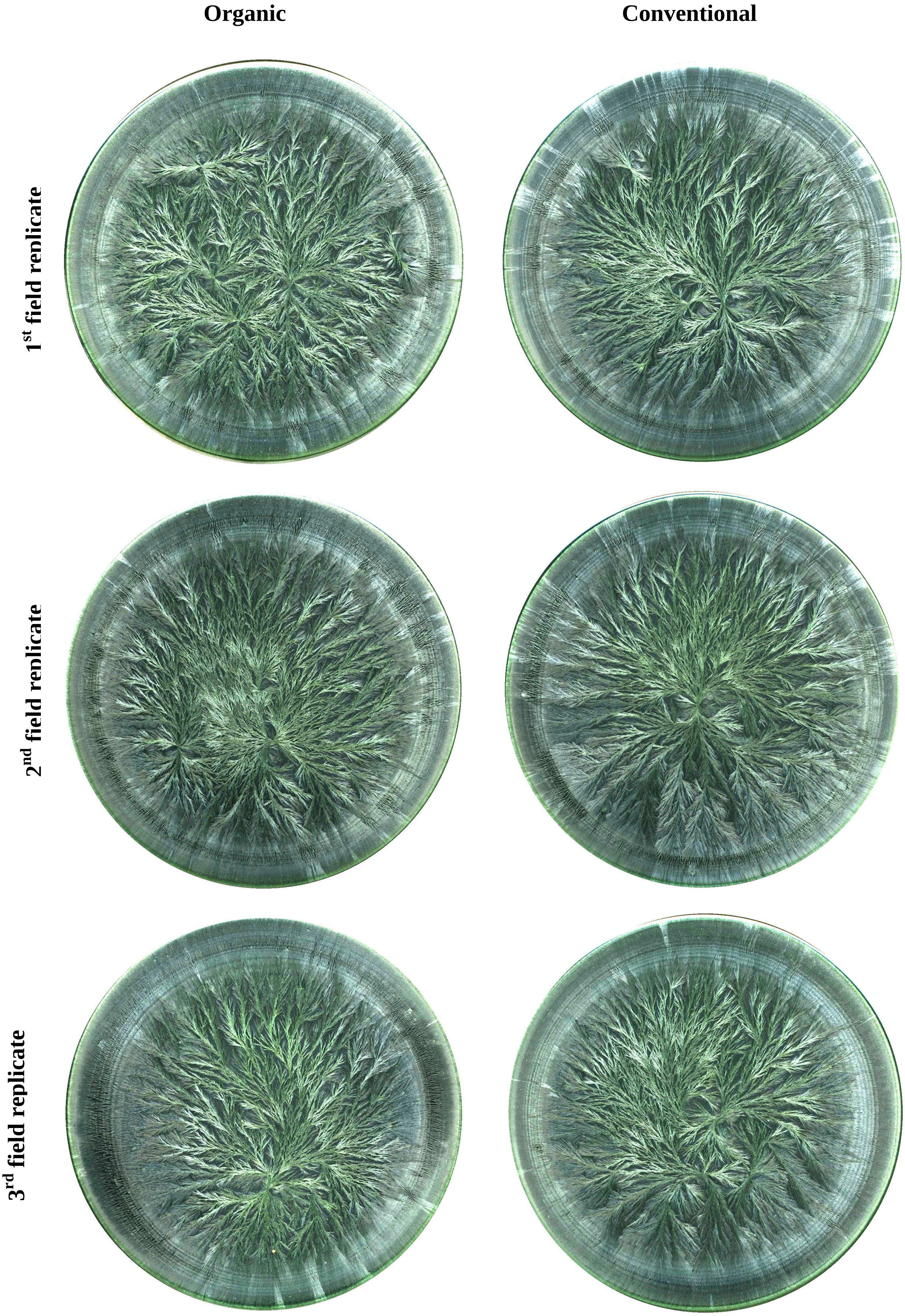
3 days old extracts



5 days old extracts



8 days old extracts



I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Kokornaczyk, Maria Olga, 30 November 2008