Technical Annex

Scientific description of the project

Title:

Content, Bioavailability and Health Effects of Trace Elements and Bioactive Components in Organic Agricultural Systems

Acronym: OrgTrace

Duration: From: Jan-2007 to Dec-2010

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English summary:

Trace elements, bioactive secondary metabolites and vitamins are among the most important quality parameters in plants. Yet, very little information is available on their content, bioavailability and health effects of organically grown plant food products. The main objective of the present proposal is to study the impact of different agricultural management practises relevant for organic farming on the ability of cereal and vegetable crops to absorb trace elements from the soil and to synthesise bioactive compounds (secondary metabolites, antioxidant vitamins and phytates) with health promoting effects. Field experiments with a rigidly controlled design will be implemented together with state-of-the-art analytical techniques allowing solid conclusions to be drawn on the variability and optimum levels of bioactive compounds such as molecular species of the elements iron (Fe), zinc (Zn), selenium (Se), sulphur (S) and bioactive metabolites (as flavonoids and carotenoids), antioxidant vitamins (vitamin C and E) and phytates. The multitude of analytical data from plant and soil samples will be analysed by multivariate statistical methods in order to reveal differences between the cultivation systems used. Finally, the relationship between bioavailability of the nutrients studied and the elemtal fingerprint of plants will be extracted by the statistical methods.

In addition to the human bioavailability study outlined above, tentative conclusions from a recently terminated study under DARCOF II will be further explored in OrgTrace. These preliminary data suggested that organic food may alter the immune status, sleep/activity pattern, accumulation of adipose tissue, liver function and vitamin E status, while traditional measures of nutrient value were unaffected by the production method of food (organic vs. conventional). Although these findings could not be directly linked to organic or conventional production systems, the observed differences were all in the favour of the organic diets. This study suffers (like most other studies reported in the literature) from the fact that only one replication per food produce was used in the animal studies, whereby the variation due to sources like field and seasonal variations, respectively, could not be estimated. In addition, it was not possible to relate the responses of humans and animals to the diet composition due to the limited number of replicates. Thus, it is of outmost importance that future investigations on the effect of organic food on human health and well-being should be based on a well-defined and well-controlled food produce system with replications of the food production systems – such as described in the current OrgTrace project.

The systematic approach of OrgTrace will be a major improvement compared to previous studies, where organic and conventional foods have been compared on the basis of a relatively narrow selection of compounds with limited information about agricultural practice and growth history. From a practical point of view, the project will generate knowledge valuable for the future plant growth optimization, nutritional quality and traceability of organically-grown foods. This will facilitate further developments in competitiveness and market share of organic foods.

A.0 Introduction, state of the art and objectives of the project:

Introduction

Quality and safety of food are important issues, which receive increasing attention in the general public. The consumption of organic foods has been steadily increasing during the last decade, particularly in Western countries (Schifferstein & Ophuis 1998; Mann 2003).

Many consumers perceive that organic foods are of better quality, healthier and more nutritious than food produced using conventional methods. However, conclusive research on possible impacts on animal and human health is sparse (Williams 2002). Moreover, the majority of studies reported in the literature are outdated because the practices in both organic and conventional agriculture have changed over time. According to a Danish knowledge-synthesis, reviewing the scientific literature (O'Doherty Jensen et al. 2001), there is no scientific evidence which can support or refute such consumer perceptions. However, a well controlled experiment conducted under "DARCOF II" showed that rats responded differently when eating complete diets of conventional and organic food produce. The results support the need for further investigations on the effect of organic food products on the health of humans. As organic products increase their market share and become more visible on the market, a polarisation of the debate occurs: Proponents of organic agriculture and those of conventional agriculture accuse each other of using unsubstantiated claims about risks or benefits in an attempt to promote their own commercial interests. Consequently, it is in the interest of both consumers and policy makers to have access to independent scientific results, which can be used to secure focus and improve the quality of the debate as well as providing foundation for possible nutritional recommendations by the authorities.

A large number of studies have addressed the question "whether organic food is more beneficial for health than conventional one". Most of these studies measure the content of well-known vitamins or minerals in plant foods of more or less controlled origin and conclude that there are relatively small but often significant differences. There are reasonably consistent findings for higher nitrate and lower vitamin C contents in conventional vegetables (amongst others Woese et al. 1997). However, since the optimal dietary intakes are still unknown both for nitrate (McKnight et al. 1999) and for vitamin C (Benzie 1999), it is not yet possible to extrapolate from such composition differences in the food to possible effects on health. Other studies indicate that the most systematic differences between organic and conventional crops are the contents of secondary metabolites (Brandt & Mølgaard 2001) or minerals (Gundersen et al. 2000).

Data concerning impacts on animal and human health of diets comprising organic or conventional produce are extremely sparse (Williams 2002). A recent study (Finamore et al. 2004) concluded that conventional wheat represented a higher risk for decreased lymphocyte function than those grown organically. In addition, the growing conditions of fruits and vegetables (conventional vs. organic) affected the content of five selected flavonoids and resulted in differences in the urinary excretion of major dietary flavonoids that are markers of oxidation in humans (Grinder-Pedersen et al. 2003).

In a recent study conducted under "DARCOF II" we investigated, through a well controlled animal feeding experiment, whether conventional and organic food products showed differences in animal physiology of a type and magnitude that indicate whether organic products would affect humans differently. In one of the sub-projects (Lauridsen et al., 2005), a rat-feeding experiment was performed comparing the effect of three iso-energetic and iso-nitrogeneos diets composed of vegetables (potatoes, carrots, peas, green kale, apples) and a high content (13%) of rapeseed oil, produced according to each of three cultivation systems on a range of physiological responses (utilisation of nutrients, immunity, anti-oxidative defence, and health status). The primary but still tentative conclusion from this study was that the most significant effects on rats of diets was observed on health aspects that have rarely been assessed in previous studies: Immune status, sleep/activity pattern, accumulation of adipose tissue, liver function, and vitamin E status, while a large numbers of markers of "traditional" nutrient value showed no differences. Even though the results of the present study could not directly be applied to organic and conventional production systems, and because most variables showed no differences between the experimental diets, the observed differences were all in favour of the organic treatment and thus pointed in the direction of potential health benefits when eating organically grown rather than conventionally grown food.

Previous Danish studies (Grinder-Pedersen et al., 2003; Lauridsen et al., 2005) have the advantages of using complete diets rather than single food items, and the diets were analysed chemically in order to explain the obtained effects on humans and rats, respectively, to the nutritional differences between the organically and conventionally diets. However, these studies like the study by Finamore et al. (2004) suffer from the fact that only one replication per food produce was used in the animal studies, i.e., the variation due to sources like field and seasonal variations, respectively, could not be estimated. In addition, it was not possible to correlate the responses of the humans and animals to the diet composition due to the limited number of replicates. Thus, there is an urgent need to systematically relate the chemical composition of diets with the bioavailability of selected bioactive components in humans and to deepen our knowledge about the health impacts of food products produced in different agricultural systems.

State of the art

Minerals and Bioactive Molecular Species

The dietary intake of certain essential trace elements via conventionally produced food is low in parts of the Danish population (Levnedsmiddelstyrelsen, 1995; Larsen et al. 2002). This is particularly the case for Fe, Zn and Se. Similarly, clinical trials in highly developed countries like the USA, New Zealand and in Europe indicate that large population groups may have a low status with respect to essential trace elements (Gibson et al., 1997; Prasad, 1998; Welch and Graham, 2004). The causes of Fe deficiency are low Fe intake, low Fe bioavailability, increased Fe requirements during periods of growth, and unusually high menstrual losses linked to methods of contraception. Lower socioeconomic groups often consume less muscle foods, which are the best source of bioavailable Fe due to the presence of highly absorbable haem Fe, and a factor enhancing the absorption of non-haem Fe (Carpenter and Mahoney, 1992). Although vegetarian diets may be relatively high in non-haem Fe, this Fe is often poorly absorbed due to the high levels of phytates (inositol-phosphates) in legume seeds and cereal grains. The current dietary recommendations to prevent chronic diseases, such as coronary heart disease and cancer, are to decrease consumption of animal source foods and increase consumption of fruits, vegetables and cereals. Fe nutrition in industrialized countries could thus deteriorate further. Due to the restricted import of fertilisers to the organic cultivation practice, as well as the inherent recirculation of plant or animal nutrients, there exists a risk of an even lower content of the trace elements in the organic cultivation practice. Therefore, there is a need for optimisation of the organic cultivation system with respect to the content of the three trace elements while at the same time maintaining the integrity of the organic cultivation practice.

The prevalence of Zn deficiency is thought to mirror Fe status. This is because muscle foods provide most of the bioavailable Zn intake in industrialized countries and because phytic acid in cereals and legume seeds is a potent inhibitor of Zn absorption. Zinc deficiency in humans reduces the immune defence system and increases the incidence of several skin diseases (Prasad 1996).

Cereal grains and vegetable plants such as *Cruciferacae* and *Allium* species are important sources of molecular species of Se and S, which act as potent bioactive antioxidants (Tapiero et al. 2003) and can help the body to fight cancer and heart diseases (Wei et al. 2004; Berges et al. 2004). Furthermore, because Se and S exhibit closely related phytochemistry, the study of their speciation and competitive interaction in the organic cultivation practise is of importance. A deficiency in Se has also been linked to adverse effects on immunocompetence (Rayman 2002) and it has recently been shown that low Se status is associated with the occurrence of the pregnancy disease preeclampsia in women from the United

Kingdom (Rayman et al. 2003). In the context of these effects, there are now concerns that dietary Se intake is inadequate for the population in the UK and other parts of Europe (Arthur 2003). As for Fe and Zn, there are marked genotypic variations in the Se concentration in wheat grains (Lyons et al. 2003) and several rhizosphere processes are involved in Se uptake by roots (Fox et al. 2003) which strongly emphasize the importance of studying various cropping systems.

The knowledge about the content and bioavailability of trace elements in organically produced foods is very limited. Gundersen et al. (2000) analysed the content of a variety of trace elements in organically grown crops, but did not include Fe, Zn and Se due to analytical constraints. The results indicated a relationship between trace element content and cultivation practices. However, factors such as soil type, crop rotation, fertilisation and soil amendments were not investigated. Furthermore, the genotype of the cultivated crop plants were not defined, which may explain some of the variation in the data on trace element concentrations.

Bioactive Secondary Metabolites, Vitamins and Phytates
Several studies have shown that a high intake of fruit and vegetables is associated with a lower risk of heart diseases and cancers (Nordic Nutrition Recommendations, 2004). The health protective effect seems to be connected to different bioactive compounds, like secondary metabolites and vitamins, in the plants (Hu, 2003). The biological role of secondary metabolites in plants is generally to protect against attack e.g. by microorganisms and insects, and accordingly, contents of secondary metabolites tends to be higher in organically than in conventionally grown crops (Grinder-Pedersen et al., 2003). Thus besides variety, contents of bioactive metabolites in plants are influenced by cultivation system, and likewise the contents of vitamins in plant species are to a large extent dependent upon genotype, but might also be influenced by cultivation practises (Leth et al 2001, Knuthsen et al, submitted).

The bioactive compounds of plants constitute many different groups, e.g. flavonoids, carotenoids, and vitamins. Looking at the health promoting effects of plants, the interest is focused at plant constituents with antioxidant effects, as cancers and heart diseases are associated with oxidative stress. The antioxidants of fruit and vegetables contribute to the antioxidative defence system, eliminating free radicals and excreting toxic compounds (Nordic Nutrition Recommendations, 2004). Consequently, in the present proposal the study will comprise bioactive compounds with antioxidant properties, like flavonoids and other polyphenols, carotenoids, glucosinolates, and the vitamins C and E. Furthermore vitamin C plays a major role for the absorption of trace elements in humans.

The individual groups of bioactive metabolites might be found in many different forms in plants, the actual forms and contents being influenced by cultivation and harvesting conditions, besides genotype of the plant, e.g. as reported for carotenoids and flavonoids in spinach (Kidmose et al 2001).

The actual binding forms of the bioactive compounds influence the activity of secondary metabolites and vitamins. It has e.g. been demonstrated that flavonoids in the glucosidic form are better absorbed in humans than other forms (Day et al, 1998, Gee et al 2000). It is thus important to investigate the many different forms of the compounds in plants, and their human bioavailability as well.

As the pattern of bioactive compounds differs from one crop to another, in OrgTrace a careful selection of compounds to be included in the investigations is made for each crop, based on health evaluations and occurrence.

It has been observed that some bioactive metabolites can inhibit or promote the absorption of trace elements (Welch and Graham, 1999), but investigations of correlations to cultivation systems are sparse. A similar property has been ascribed to the natural content of phytates, which are responsible for the storage of minerals in plant seeds. Although being of importance in the homeostasis of minerals in plants, the same constituents are responsible for the poor utilisation of minerals in human and animal nutrition. Several investigations have shown that organically-grown cereals are lower in phytate and up to 60 % higher in Zn than their conventionally cultivated counterparts. This has been ascribed to the absence of readily soluble phosphate fertilisers in cultivation (Graham et al. 2000; Ryan et al., 2004). The significant increase in the Zn content in the kernel and the higher Zn/phytate ratio makes it likely that organically-grown cereals may have improved mineral bioavailability and, consequently, nutritional value. This important aspect needs to be confirmed in a Danish context. The OrgTrace project will generate important knowledge about the impact of cultivation systems on the mineral and bioactive compounds in plants, as well as their impact on health aspects by the human intervention studies.

Objectives

The overall scope of the proposal is to improve the fundamental knowledge of organically grown foods in order to further document their quality in relation to agricultural practice. This will be realised in the present project by investigating the influence of a variety of rigidly controlled cultivation methods on the content of bioactive compounds of importance in human nutrition as well as for promotion of human health.

The underlying hypothesis is that a careful choice of crop rotation and cultivation practice will allow for an organic crop production system characterised by crops of superior nutritional quality with respect to trace elements, bioactive metabolites and vitamins. Phytate, being a potential counteracting substance will also be analysed. Such an optimised cultivation system will respect and ensure the integrity and efficiency of the organic cultivation practice in all links of the chain from the consumer to the primary production.

The specific objectives are:

1. To screen the content of trace elements together with other relevant bioactive constituents in a wide selection of commonly consumed organic crops. Yet unidentified molecular species of bioactive compounds in the organically grown crops will be further examined if they are quantitatively significant. Multivariate methods of data analysis will be employed to differentiate between the agricultural systems.
2. To characterise and optimise the content of trace elements and bioactive compounds in crop plants harvested from a well-defined organic cultivation system including realistic combinations of crop species, green manures, catch crops, soil type, crop rotations and organic fertilizers.
3. To assess the bioavailability of the bioactive compounds in human intervention studies employing prepared foods based on the cultivated crops.
4. To study the effects of foods on health and well being after long-term consumption using the rat as a model.

A.1 Technical content of the research activity

Work Package list

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WP No.	WP title	Responsible scientist	Budget DKK	Start	End	Deliverable No.
1	Multielemental analysis of plant and soil, multivariate data analysis and project coordination	Søren Husted	2301	01-2007	12-2010	D1.1-D1.5
2	Identification of major bioactive plant constituents	Pia Knuthsen	1969	01-2007	12-2009	D2.1-D2.8
3	Sample pre-treatment and analysis of crops	Erik H. Larsen	2380	06-2007	12-2009	D3.1-D3.6
4	Health and immunity elucidated by a rat model	Charlotte Lauridsen	4000	08-2007	12-2010	D4.1-D4.4
5	Human bioavailability	Susanne Bügel	3737	05-2007	12-2010	D5.1-D5.5
Total			**14.387**			

(Please give month and year for start and end)

Deliverables list

Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person months	Type of deliverable
D1.1	Multi-elemental classification of organic and conventional agricultural systems	SHu	12–2010	12	S
D1.2	Mobilization of Se and S by green manures and catch crops in organic agriculture	SHu	12–2010	6	S
D1.3	"Er der forskel på økologisk og konventionelt dyrkede planter i Danmark" Popular scientific contribution in Danish	SHu	12-2010	0	P
D1.4	Presentation of primary data at a suitable international congress	SHu	08-2010	0	C
D1.5	Ph.D thesis - Multi-elemental classification of crops from organic and conventional agricultural systems using inorganic mass spectrometry and multivariate statistics	SHu	12–2010	4	O
				22	
D2.1	Presentation of results on identification/characterization of selected unknown bioactive trace elements at international symposium	EHL	02-2008	2	C
D2.2	Presentation of results on identification/characterization of selected unknown bioactive secondary metabolites at international symposium	PK	12-2008	3	C
D2.3	Paper on S and Se speciation in crops	EHL	06-2008	8	S
D2.4	Paper on characterization of selected bioactive metabolites in crop plants	PK	10-2008	9	S
D2.5	Ph. D thesis on S and Se in crops and diets, and their bioavailability	EHL	12-2009	3	O

D2.6	Ph. D thesis on bioactive metabolites in crops and diets, and their bioavailability	PK	12-2009	3	O
D2.7	Identification of key Fe and Zn species in cereal grains grown in different agricultural systems	SHu	12–2010	8	S
D2.8	Ph.D thesis – Speciation and bioavailability of Fe and Zn in the cereal grain (see also D3.6)	SHu	12-2010	4	O
				40	
D3.1	Paper on ^{74}Se enrichment of crop plants via foliar application	EHL	04-2009	7	S
D3.2	Paper on composition of bioactive metabolites and vitamins in organic and conventional crops	PK	07-2009	11	S
D3.3	Presentation of results on D3.2 at international conference	PK	08-2009	3	C
D3.4	Paper on results for Se and S species in conventional and organic crops	EHL	12-2009	12	S
D3.5	Speciation of Fe and Zn in cereal grains grown in different agricultural systems	SHu	12–2010	8	S
D3.6	Ph.D thesis – Speciation and bioavailability of Fe and Zn in the cereal grain (se also D2.8)	SHu	12-2010	7	O
				48	
D4.1	Differences among cultivation systems with respect to the development of the immune function	CLA	12-2010	18	S
D4.2.	The effect of cultivation systems with respect to health, antioxidant and nutritional status, and physical activity	CLA	12-2010	18	S
D4.3.	Preference test between diets of different cultivation systems	CLA	12-2010	10	S
D4.4	Ph.D. –thesis with the suggested title: "Influence of organic food on health using the rat as a model for humans"	CLA	10-2010	4	O
				40	
D5.1	Presentation of results at international conference	SHB	12-2010	0	C
D5.2	Draft Scientific publication "Bioavailability of micronutrients from different organic production systems in humans	SHB	12-2010	14	S
D5.3	Draft Scientific publication "Bioavailability of bioactive metabolites from different organic production systems in humans	SHB	12-2010	14	S
D5.4	Draft Scientific publication: Interaction between micronutrients and bioactive metabolites from different production systems in humans	SHB	12-2010	14	S
D5.5	Ph.D thesis	SHB	12-2010	4	O
				46	

(The nature of the deliverables must be indicated by S = publication in scientific journal with peer review; P = publication in journals without peer review; R = reports; C = presentation at meetings and congresses or O = other types of deliverables, e.g., prototypes, models, websites, etc.).

Milestones list

Mile-stone No	Milestone title	Lead scientist	Delivery date
M1.1	Classification of soil based on data from sequential extraction (year 1)	SHu	07-2007
M1.2	Classification of multi-elemental data from plant samples (year 1)	SHu	03-2008
M1.3	Classification of soil based on data from sequential extraction (year2)	SHu	07-2008
M1.4	Classification of multi-elemental data from plant samples (year 2)	SHu	03-2009
M1.5	Multivariate data analysis	SHu	12-2010
M2.1	HPLC and ICP-MS systems optimised for simultaneous determination of Se and S species	EHL	09-2007
M2.2	LC, LC-MS and LC-MS-MS methods tailored for determination of selected bioactive metabolites in crops	PK	12-2007
M2.3	Selected unknown trace element species characterized or identified	EHL	12-2007
M2.4	Selected unknown bioactive metabolites characterized or identified	PK	01-2008
M2.5	Key Fe and Zn species identified	SHu	01-2008
M3.1	Crops 2007 analysed for S and Se molecular species	EHL	04-2008
M3.2	Crops 2007 analysed for selected bioactive metabolites and vitamins	PK	04-2008
M3.3	Speciation analysis of Fe and Zn in cereal grains (year 1)	SHu	08-2008
M3.4	Crops 2008 analysed for S and Se molecular species	EHL	04-2009
M3.5	Crops 2008 analysed for selected bioactive metabolites and vitamins	PK	04-2009
M3.6	Speciation analysis of Fe and Zn in cereal grains (year 2)	SHu	08-2009
M4.1	Complete diets of ingredients from Cropsys of cultivation year 1 are prepared	CLA	01-2008
M4.2	Analytical methods regarding the development of the immune function of rats are available	HRJM	02-2008
M4.3	Rat experiments including chemical analyses of cultivation year 1 is performed	CLA	01-2008
M4.4	Complete diets of ingredients from Cropsys of cultivation year 2 are prepared	CLA	01-2009
M4.5	Rat experiments including chemical analyses of cultivation year 2 are performed	CLA	01-2010
M4.6	Overall data-analyses are performed	CLA	07-2010
M5.1	Ethical approval obtained	SHB	05-2007
M5.2	Production of diets for the intervention study, year 1, including the stable isotope labelled diet	SHB	10-2007
M5.3	Recruitment of study population for the 1.year intervention study	SHB	10-2007

M5.4	Complete diets for human and rats of 2007 analysed for Fe, Zn, Se and S	EHL	02-2008
M5.5	Complete diets for human and rats of 2007 analysed for bioactive metabolites and vitamins	PK	04-2008
M5.6	Diet (2007) analysed for phytates	SHu	05-2008
M5.7	Completion of dietary intervention study year 1	SHB	06-2008
M5.8	Collection and distribution of human samples from 1.year intervention study	SHB	09-2008
M5.9	Analyses of human samples from 1.year intervention study	SHB	02-2009
M5.10	Production of diets for the intervention study, year 2	SHB	10-2008
M5.11	Recruitment of study population for the 2.year intervention study	SHB	10-2008
M5.12	Urine, plasma and faeces (2007 harvest) analysed for Fe, Zn and Se and enriched stable isotopes	EHL	02-2009
M5.13	Urine and plasma (2007 harvest) analysed for bioactive metabolites and vitamins	PK	02-2009
M5.14	Complete diets for human and rats of 2008 analysed for Fe, Zn, Se and S	EHL	02-2009
M5.15	Complete diets for human and rats of 2008 analysed for bioactive metabolites and vitamins	PK	04-2009
M5.16	Diet (2008) analysed for phytates	SHu	05-2009
M5.17	Dietary intervention study year 2 completed, J	SHB	06-2009
M5.18	Collection and distribution of human samples from 2.year intervention study,	SHB	09-2009
M5.19	Analyses of human samples from 2.year intervention study	SHB	02-2010
M5.20	Estimation of absorption of trace elements	EHL	06-2010
M5.21	Urine, plasma and faeces (2008 harvest) analysed for Fe, Zn and Se and enriched stable isotopes	EHL	02-2010
M5.22	Urine and plasma (2008 harvest) analysed for bioactive metabolites and vitamins	PK	02-2010
M5.23	Estimation of absorption of bioactive metabolites	PK	06-2010
M5.24	Multivariate data analysis of data	SHu	12-2010

Description of work packages

WP No.: 1 Multi-elemental classification analysis of plant and soil, multivariate data analysis and project coordination

	Start date or starting event: 01.01.2007								
Partner id.	KVL-PSSL								
Person-months per participant	22								
Total PM:	**22**								

(Please give Institution or Department as partner id.)

Objectives:

- Sample logistics and project coordination
- Multi-elemental classification of plant and soil according to agricultural system and geographical origin
- The effect of green manures and catch crops on the mobility of Se and S in different agricultural systems
- Multivariate analysis of analytical data

Description of work:

Task 1: Project coordination

The project coordinator is responsible for contact to the project leaders of Cropsys and VegQure. On a regular basis the field trials (see below) will be supervised in order to monitor field performance and to ensure that the plant material is of sufficient quality for the experiments in OrgTrace. The project coordinator is responsible for collection of harvested products and distribution to the individual partners of OrgTrace.

The project will be managed by a Project Steering Committee (PSC) consisting of the leaders of the individual WP's and chaired by the project leader. At the PSC meetings, progress reports on each WP will be presented according to the milestone and deliverable table and any adjustments of the initial plan will be decided. Any possible emerging scientific and technical obstacles will also be discussed and the work plan will be adjusted accordingly. At these meetings also the progress reports to be delivered to DARCOF III will be discussed. The status of planned publications will be reviewed and an updated publication plan will be decided.

The project coordinator also ensures the mandatory popular contributions to FØJOnyt, DARCOFenews and columns ("klummer"). These tasks will be distributed equally between the individual WP's.

A Gantt table is attached as an MS-EXCEL file. This table describes the timeline for all milestones and deliverables in OrgTrace

Task 2: Plant cultivation.

All plant products used in OrgTrace will be supplied from the projects Cropsys and VegQure. However, collection of harvested products at the geographical locations, sample pre-treatment and storage is undertaken by OrgTrace.

In the growing season 2007 and 2008 plant and soil samples will be analysed according to the following schemes (Table 1 & 2):

Table 1: Crop rotations included in OrgTrace

Diet	Agricultural system	Cropsys abbreviation	VegCure abbteviation	Notes
1	Organic A	O4; -CC/+M	O1; slurry	Slurry application
2	Organic B	O4; +CC/-M	O2; green manure	Green manure and catch crops
3	Conventional with NPK fertilizer and pesticide application	C4; -CC/+M	C1; NPK	Inorganic fertilizers

Table 2: Crops species, number of replicates and geographical locations of the crop rotations used in OrgTrace.

Crops	Project	Season	Replicates	Locations
Carrot	VEG-QURE	2007/2008	3	Årslev
Onion	VEG-QURE	2007/2008	3	Årslev
White Cabbage	VEG-QURE	2007/2008	3	Årslev
Oats	VEG-QURE	2007/2008	3	Årslev
Spring barley	CROPSYS	2007/2008	2	Jyndevad, Fou-lum, Flakkebjerg
Potato	CROPSYS	2007/2008	2	Jyndevad, Fou-lum, Flakkebjerg
Wheat	CROPSYS	2007/2008	2	Jyndevad, Fou-lum, Flakkebjerg
Bean	CROPSYS	2007/2008	2	Jyndevad, Fou-lum, Flakkebjerg

In addition to the plants listed in Table 2 oil-seed rape will be produced as a fat-source for the rat and human diets. This implies that the following number of samples will be analysed in each of the growing seasons 2007-2008:

Cropsys: (Crop x Location x System x Replicate) = 4 x 3 x 3 x 2 = 72 units

VegCure: = 4 x 1 x 3 x 3 = 36 units

Total per growing season = ***108 units*** *to be analysed per year*

Task 3: Plant sampling and multi-elemental classification analysis:

Plant products will be sampled when they are ready for harvest – this process will be continuously running in the period from August to November 2007 and 2008. Sampling and initial preparation of the plant products will be undertaken by the field personnel at the different locations. The coordinators for Cropsys and VegQure will contact the coordinator of OrgTrace, being responsible for distribution of material to the partners of the project, when the plant products are ready for harvest. Each replicate is being kept separately – this is of vital importance for the data analysis. Decontamination of the plant surface, which is vital to this project, will be undertaken at KVL in the dedicated kitchen facilities at IHE.

The decontaminated samples are freeze-dried and milled in a titanium mill. Digestion of plant material is undertaken in a microwave oven. Multi-elemental analysis will be performed according to principles outlined in Husted et al. (2004).

The following elements will be analysed (Table 3):

Table 3: Nutrients to be analysed by ICP-OES, ICP-MS and IR-MS. The elements listed in grey will be analysed by IR-MS providing isotope ratios of the elements.

Essential Plant macro nutrients	C	N	P	K	Mg	Ca	S
Essential Plant Micro nutrient	Fe	Mn	Cu	Zn	Ni	Mo	B
Non-Essential Plant Nano nutrients	Se	Co					
Contamination markers	Al	Ti					

In addition to the 18 elements listed in Table 3, a semi-quantitative analysis will be performed on a selection of the samples in order to identify 20-40 additional elements with significant ion intensities allowing us to perform a quantification above the instrumental detection limit (LOD). A total number of approximately 40 elements are needed for each sample in order to perform the statistical classification of the agricultural systems as described below.

A quality assurance program will be developed which qualifies the samples according to LOD and accuracy. Accuracy is measured relative to a suitable certified reference material (CRM) and in addition an in-house reference will be selected which represent the concentration ranges of the elements to be considered. These reference samples will run in parallel with the crop samples and only samples with accuracy better than 90% will be accepted. Al and Ti will be used as contamination markers as the ion intensities of these elements usually are low in plant tissue unless surface contaminated with dust and soil.

The photochemical efficiency (Chl *a* fluorescence) will be measured in all cereals of Cropsys in order to related the general vitality of plant samples with the multi-elemental composition. This analysis will be undertaken in april 2007 and 2008.

Task 4: Soil sampling and analysis:

The influence of crop rotation on basic soil characteristics will be monitored thoroughly because these data are essential in order to understand the chemical imprint that is being analysed in the plant tissues. Before each growing season in early spring, the soil in all 108 plots will be sampled and analysed according to the following scheme (Husted et al. 2004). :

Table 4. Overview on the extractants used to sequentially extract selected elements from the soil samples.

Extractant		Special relevance for:	Common abbreviation
1	Hot ultra pure water	All elements selected in WP1	Bt
2	50% HNO_3	All elements selected in WP1	--
3	Ammonium acetate (0.5M)	K, Na, Mg, Ca	Kt, Mgt
4	Bicarbonate (0.5 M pH=8.5)	P, S, Se	Pt
5	0.02 M EDTA/0.1 M NH_4Cl	Cu	Cut
6	DTPA/TEA/$CaCl_2$	All cationic transition elements (X)	DTPA-X
7	Ammonium-Oxalate (pH 3.3)	Mo	Mot

Note: Critical and potential toxic elements such as Cd and Pb will also be included in the analysis.

In addition to these measurements we will have soil key-parameters such as: Rt, texture and SOM analysed in cooperation with Cropsys and VeqQure. These analyses will be outsourced to a certified private lab.

Samples will be sampled before the onset of the growing season in 2007 and 2008 (March-April) and sampling will follow a random walk or diagonal walk procedure where 15-20 sub-samples taken with a titanium auger (25 cm) in each plot are pooled to one composite sample. It is important that these samples are taken before the spring application of slurry in order to avoid the impact of deposited elements on the soil surface.

At the Årslev location (VegQure) we will analyse the ability of deep rooted and Se-accumulating green manures (GM) to mobilize S and Se from deep soil layers to the top-soil. This is done by sampling soil from sequential 20 cm layers ranging from 2.4 m soil depth to the top-soil. Soil sampling will be undertaken according to principles previously developed at the Årslev location. By comparing the extractable amount of Se in these plots (designated O2) with the reference plot (designated C1) essential information on the importance of green manures on the mobility and bioavailability of Se in organic farming is obtained. These experiments will be planned and undertaken in close cooperation with the project leader of VegQure.

Task 5: Multivariate data analysis (Chemometrics)

The main objectives of OrgTrace are to study the impact of different agricultural practises on the ability of selected plants to absorb inorganic elements and to synthesise bioactive compounds. Furthermore, the human bioavailability of selected key-candidates and the health impacts on rats will be assessed (WP4-5). These objectives will be fulfilled by analysing the multi-elemental fingerprint of trace elements and of selected organic bioactive metabolites of which several are known to be health promoting and some are believed to have adverse effects on the human bioavailability of essential elements (WP2 and WP3). The trace elements are analysed by modern state-of-the-art ICP based analytical equipment and the bioactive metabolites are analysed by organic mass spectrometry such as LC-MS-MS. This equipment produce data matrices of a true multivariate nature and a comprehensive data mining can only be achieved by applying principal component analysis (PCA) and other multivariate data analysis techniques. The purpose of PCA is to decompose the data in order to analyse the hidden structure of the data matrices and extract the significant patterns.

In this project PCA will be used to analyse the analytical data from the soil characterization and plant tissue analyses in order to identify the impact of crop rotations and the different agricultural practises on fingerprints of trace elements and bioactive components. Principal component analysis will allow identification of the effects of the selected green manures in comparison with slurry on concentrations of trace element and bioactive components in crops and link soil characteristics to the plant tissue composition. Using PCA on the data material obtained in this study will provide fundamental insight on the levels of some of the most important components determining plant quality of organically-grown cereals and vegetables. In addition it will provide information about ways to optimize the content of compounds with health promoting characteristics in organic farming.

Principal component analysis is also ideally suited to provide information about the parameters which control the bioavailability of trace elements and bioactive metabolites in humans. The analysis will include all data generated in the project (plant, soil, diets and human bioavailability data) and group parameters according to their ability to promote human absorption (WP5). This kind of analysis will be a major improvement compared to most studies undertaken so far, where usually a relatively limited selection of typically health promoting compounds in organic grown plants have been analysed and compared with a test set of conventional products.

In this project we intend to use the true multivariate data matrices, generated by the comprehensive amount of trace elements and bioactive metabolites included, to develop a multivariate classification model based on SIMCA (Soft Independt Modelling of Class Analogy). This multivariate model will allow a unique comparison of the organic crop species included in this study with any collection of similar crop species originating from conventional farming systems or other organically farming systems. The multivariate model will be based on the multitude of trace elements and bioactive metabolites included in the comprehensive field studies being the backbone of the OrgTrace project.

Deliverables:

D1.1 Multi-elemental classification of organic and conventional agricultural systems
D1.2 Mobilization of Se and S by green manures and catch crops in organic agriculture
D1.3 "Popular Scientific Contribution"
D1.4 Presentation of data at an international conference
D1.5 Ph.D thesis (Multi-elemental classification)

Milestones:

M1.1 Classification of soil based on data from sequential extraction (year 1)
M1.2 Classification of multi-elemental data from plant samples (year 1)
M1.3 Classification of soil based on data from sequential extraction (year2)
M1.4 Classification of multi-elemental data from plant samples (year 2)
M1.5 Multivariate analysis

Description of work packages (this page should be copied for each WP**)**

WP No.: 2 Identification of major bioactive plant constituents

	Start date or starting event: 01.01.2007								
Partner id.	KVL-PSSL	DFVF-K1	DFVF-K2						
Person-months per participant	12	17	15						
Total PM:	**44**								

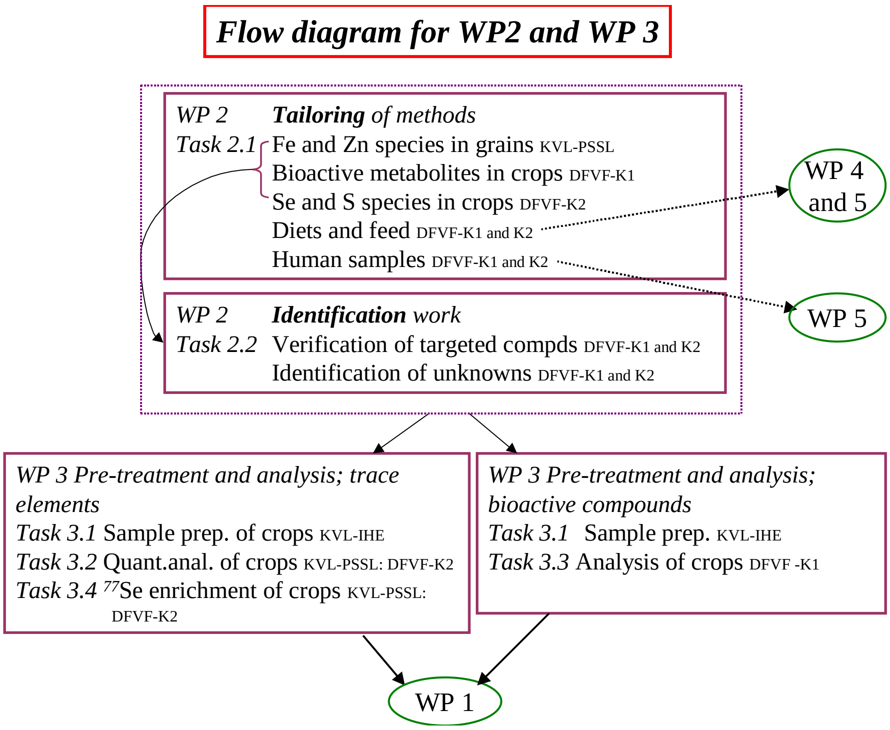
Objectives:

- Tailoring of existing analytical methodologies to determination of trace element species and bioactive secondary metabolites in crops, including development of species-conserving methods of analysis
- Chemical characterization or identification of trace element species of unknown identity
- Identification of molecular species of Fe and Zn in cereal grains produced in different agricultural systems
- Chemical characterization or identification of bioactive metabolites and determination of antioxidant vitamins in crops

Description of work:

Spring barley, bread wheat, white cabbage, carrot, onion, potato, and horse beans grown during the 2-year field experiment (WP1) will be characterized in WP2 and WP3. Suitable analytical methods will be developed and validated and key-components will be identified in WP2. This knowledge will used to screen crops for bioactive components with health promoting effects in WP3.

In order to carry out the WP3 analyses in a precise and accurate manner, and foreseeing that some bioactive compounds of unknown identity will be present, this work package aims at developing and implementing the analytical methodologies necessary for a successful completion of WP3.
Furthermore WP2 will establish the basis for analysis of bioactive compounds in feed for rats in WP4, as well as diets and human samples in WP5. The flow diagram for the analytical method developments and identification work is illustrated below:



The main theme to be addressed in WP2 is: In addition to the occurrence of well-known and identified bioactive compounds, a number of similar other relevant compounds as well as their derivatives, will be present in the crop plants. The biosynthetic routes governed by the plant genome may cause this existence of such unforeseen natural products in the selected crop plants. Thus identification and characterisation of yet unidentified molecular species and forms of bioactive compounds will be pursued, based upon their quantitative significance or substantial equivalence.

The most cost-effective way to conduct the work described in WP2 is by involving Ph.D. students under the supervision of senior staff personnel of DFVF (professor Erik Huusfeldt Larsen and senior scientist Pia Knuthsen). Both students will be registered at KVL.
One Ph.D. student at DFVF will be engaged in the work on adaptation of existing methods to the analysis and characterisation of chemical forms of trace elements, and at KVL supervised by Assoc. Professor Søren Husted. The other Ph.D. student will be engaged with similar work on the bioactive metabolites, and supervised by Prof. John Nielsen, Biorganic Chemistry Group, Department of Natural Sciences at KVL. The fruitful interaction between the existing experiences gained by the scientists at DFVF with those of the universities where the students' academic supervisor is anchored, will provide the optimum stimulation for the students' solution of the analytical problems. Such a cooperation will also provide

access to the necessary equipment to be used for completion of the tasks.

Task 2.1 Tailoring existing methods of analysis to determination of bioactive compounds in crop plants

The selected bioactive elements will be analysed by coupled techniques employing high performance liquid chromatography (HPLC) with mass spectrometric (MS) detection. The available analytical instrumentation, which will be used for the polar constituents are HPLC-ICP-MS and HPLC-ESI-MS/MS. Special emphasis will be placed on the simultaneous determination of Se and S containing molecular species (DFVF) and on Fe and Zn species in the cereal grain (KVL). Before the chemical analysis can be carried out, the extraction and conservation of the target bioactive compounds is of importance. Therefore species conserving methods of extraction will be meticulously tested. A particular problem that needs to be addressed is the possible oxidative or enzyme-mediated degradation of S and Se-containing amino acids or peptides. Similarly, the bioactive metabolites will be analysed by employing HPLC and coupling with MS and MS-MS detection. The extraction methods preceding chemical analysis will cover different procedures for the different groups of metabolites. Polymerisation reactions of the bioactive polyphenol metabolites is a key problem that needs to be addressed and solved. Finally, the further refinement of the selectivity of existing HPLC-separation methods will be necessitated by the possible occurrence of bioactive constituents of unknown identity.

Task 2.2: Identification of bioactive trace element species and bioactive metabolites in crops

Following chemical analysis of the sample extracts by the aforementioned, coupled methods of analysis, chromatographic peaks of unknown identity are expected to occur. This means that a substance containing the trace element of interest, i.e. S or Se, or a bioactive metabolite, e.g. a flavonoid, has been detected by HPLC or GC coupled with ICP-MS or ESI-MS/MS or by HPLC-MS-MS, but does not co-elute with any of the standard substances available. Therefore, structural elucidation will be pursued by other analytical techniques including ESI-MS/MS and NMR spectrometry.

Regarding the exploitation of the latter identification technique, cooperation with the Danish University for the Pharmaceutical Sciences will be arranged (associate professor Claus Cornett). Once established, the developed methods of analysis will be characterised as to their performance in terms of precision and accuracy.

Deliverables:

D2.1	Presentation of results on identification/characterization of selected unknown bioactive trace elements at international symposium
D2.2	Presentation of results on identification/characterization of selected unknown bioactive secondary metabolites at international symposium
D2.3	Paper on S and Se speciation in crops
D2.4	Paper on characterization of selected bioactive metabolites in crop plants
D2.5	Ph. D thesis on S and Se in crops, diets and human samples
D2.6	Ph.D thesis on bioactive metabolites in crops, diets, and human samples
D2.7	Identification of key Fe and Zn species in cereals grains grown in different agricultural systems
D2.8	Ph.D thesis – Speciation and bioavailability of Fe and Zn in the cereal grain (se also D3.6)

Milestones:

M2.1	HPLC and ICP-MS systems optimised for simultaneous determination of Se and S species
M2.2	LC, LC-MS and LC-MS-MS methods tailored for determination of selected bioactive metabolites in crops
M2.3	Selected unknown trace element species characterized or identified

M2.4	Selected unknown bioactive metabolites characterized or identified
M2.5	Key Fe and Zn species identified

WP No.: 3 Sample pre-treatment and analysis of crops									
	Start date or starting event: 06/2007								
Partner id.	KVL-PSSL	DFVF-K2	KVL-IHE	DFVF-K1					
Person-months per participant	12	21	3	17					
Total PM:	**53**								

Objectives:

- Preparation of crops prior to chemical analysis
- Enrichment of crop plants with selenium via foliar application of ^{74}Se
- Chemical analysis of trace elements and element species
- Chemical analysis of bioactive secondary metabolites and vitamins

Description of work:

Work package 3 describes the sample pre-treatment and the chemical analyses for chemical characterisation of the content of bioactive compounds in the organically grown crops. The cultivation of crops have been described in WP1.

Task 3.1 Sample pre-treatment
The work on sample pre-treatment, which will be initiated by KVL-IHE, will employ techniques and methods devised by DFVF-K1 and DFVF-K2 aiming at the best possible preservation of the bioactive constituents to be determined in the crops harvested from CropSys and VegCure. More specifically, the crops for analysis of elements and species will be treated as follows:

Crop	Pre-treatment	Storage	Sub sample for chemical analysis
Wheat	Removal of husks	Dry at room temperature	Flour
White cabbage	Removal of soil contaminated outer leaves	Cool (4 °C) in the dark	Whole edible portion of crop subdivided in eigths
Carrot	Removal of adhered dirt	Cool (4 °C) in the dark	Peeled carrot root
Onion	Removal of roots and tops. Crushing in liquid N_2	Cold (-80 °C) in the dark	Freeze dried whole onion

Crops for analysis of bioactive metabolites and vitamins will comprise the vegetables cabbage, carrot, onion, horse bean, potato, and wheat and barley. Prior to chemical analysis the crops will be cleaned from soil. Preparing samples for analysis will comprise taking aliquots of 4-8 individuals of each vegetable from a certain cultivation system, in order to get representative samples. Only the edible part of the crops will be taking for analysis. Wheat and barley will be milled and stored under nitrogen until analysis. Vegetable samples will be homogenized in liquid nitrogen or in metaphosphoric acid for vitamin C, freeze-dried, and stored under nitrogen at ÷20 °C until analysis.

The cryogenic sample pre-treatment and storage of the harvested crops at +4 °C or at –80 °C will take place in existing facilities in the participating institutes.

Task 3.2 Analysis of Fe, Zn, Se and S and their bioactive molecular species
All crops will be analysed for Fe, Zn, Se and S by state-of-the-art ICP-MS instrumentation using reaction/collision cell technology for their interference-free determination. The pre-treated samples (Task 3.1) will be further homogenised using ultra-pure Ti-blender blades for prevention of contamination of the samples. The samples will be dissolved by microwave-assisted pressurised ashing with nitric acid in quarts equipment. A full QA scheme will be applied to document the limit of detection, precision (from double determinations) and the accuracy of the analyses (by the use of certified reference materials).

Based on these results, crops rich in Se and/or S will be selected for further speciation analysis. The crops will be selected on the basis of a high total concentration of the elements while at the same time ensuring that all cultivation schemes will be represented. The speciation work will employ, as relevant, aqueous extraction and/or relevant enzymes (proteases; beta-glucosidases) for liberation of the trace element species into solution. The conservation of the organometallic species is of utmost importance and will be based on methodologies developed in WP 2. The analyses will be conducted by high performance liquid chromatography (HPLC) coupled with ICP-MS supplemented by HPLC-ESI-MS for confirmation of the identity of the elemental species. Again this QA measure will rely on the results obtained from WP2.

The information gained in Task 3.2 will contribute to the data analysis to be carried out in WP1 with particular interest in the effect of the field experiments and cultivation systems used. Finally, the samples will be analysed for the occurring complexes of Fe and Zn with the natural content of phytates in the crops. This will be conducted by existing HPLC methodologies, and the results will be compared with available literature information from related studies of conventionally grown crops

Task 3.3 Analysis of bioactive secondary metabolites and vitamins and phytates
All crops will be analysed for bioactive secondary metabolites and vitamins and phytates in representative samples in accordance with the screening and the field experiment. Before determinations, the homogenized samples will be extracted by methods that have been optimised in WP2 and are carefully fitted for each class of compounds. Analyses will comprise relevant bioactive compounds, the selection of these also being based on the characterisation and optimized methodologies developed in WP2. Flavonoids will be determined in appropriate vegetables as well as corn, and individual compounds will be identified and quantified by LC-PDA, LC-MS, and LC-MSMS analysis. Carrots will be analysed for carotenoids by LC-PDA, and for falcarinols by a LC method based on (Hansen et al., 2003). Barley and beans will be analysed for proanthocyanidins, essentially by LC-MS methodology (Gu et al., 2003).

Vitamin C will be analysed be a DANAK-accredited LC-method, determining ascorbic acid as well as its oxidised form, dehydroascorbic acid, in vegetables and potato. Grains will be analysed for vitamin E by a LC-method, determining the different natural forms of tocopherols and tocotrienols. Phytates will be analysed in all crops by standard LC and LC-MS methodologies. A full QA scheme will be applied to document the precision and accuracy of the analyses.

The results and information gained in Task 3.3 will contribute to the composition of feed and diets in WP4 and WP5, respectively. After the preparation of complete diets in WP4 and WP5, the diets will be analysed for secondary metabolites and vitamins and phytates. Furthermore, the data will contribute to the data analysis to be carried out in WP1 with particular interest in the effect of the cultivation system used.

Task 3.4 Enrichment of crop plants by ^{74}Se for human bioavailability studies

In order to conduct the human bioavailability studies for selenium, the intrinsic labelling of the relevant crop plants by enriched stable Se-isotopes is mandatory. This is because selenium is covalently bound in organic selenium species biosynthesised by the crop plants. In contrast, the stable isotopes of Fe and Zn can be added extrinsically whereby the necessary isotopic equilibration is possible.

The enrichment of wheat, onion, carrot and cabbage will take place in a green house at KVL by cultivation of the same genotypes as used in CropSys and VegQure. The eniched stable ^{74}Se will be incorporated via foliar application of a ^{74}Se-selenite solution. Hereby the utilisation of the precious enriched stable isotope is highly efficient. However, we need to control that foliar application of selenium leads to the formation of the same selenium species as those biosynthesised by the plants cultivated in the field via root uptake. Therefore, pattern of selenium species in the two sets of plants will be compared using field crops from 2007. If the results confirm identical patterns of selenium species, the enriched plants are fit for purpose in the human intervention studies. If the pattern of Se-species detected in crops using foliar application differs markedly from field crops, the relevant isotope enriched Se species will be synthesised and used in the human studies.

Deliverables:

D3.1	Paper on ^{74}Se enrichment of crop plants via foliar application
D3.2	Paper on composition of bioactive metabolites and vitamins in organic and conventional crops
D3.3	Presentation of results on D3.2 at international conference
D3.4	Paper on results for Se and S species in conventional and organic crops
D3.5	Speciation of Fe and Zn in cereal grains grown in different agricultural systems
D3.6	Ph.D thesis Speciation and bioavailability of Fe and Zn in cereal grains (See also D2.8)

Milestones:

M3.1	Crops 2007 analysed for S and Se molecular species
M3.2	Crops 2007 analysed for selected bioactive metabolites and vitamins
M3.3	Speciation analysis of Fe and Zn in cereal grains (year 1)
M3.4	Crops 2008 analysed for S and Se molecular species
M3.5	Crops 2008 analysed for selected bioactive metabolites and vitamins
M3.6	Speciation analysis of Fe and Zn in cereal grains (year 2)

Description of work packages (this page should be copied for each WP**)**

WP No. 4:	Effect of cultivation systems on health status of rats

	Start date or starting event: 1/8-2007								
Partner id.	DIAS, SVE	DIAS, GBI							
Person-months per participant	70	1							
Total PM:	**71**								

(Please give Institution or Department as partner id.)

Objectives:

- To design complete diets according to cultivation systems as described in CROPSYS
- To characterise how biomarkers for health and well-being are affected by the complete diets using a rat model
- To study the development of the immune defence system according to the dietary treatments
- To investigate the choice of diets by rats in a preference test

Description of work:

Task 1: Complete diets (18 per year; 2 cultivation years) will be prepared from ingredients (barley, wheat, potatoes, horse bean and rapeseed) obtained from the CROPSYS-project. The sub-plots involve cultivation systems (N=3), site of cultivation (N=3), and repetition (N=2). The ingredients will be prepared before mixing the diets: Potatoes will be freeze-dried, meal will be prepared from grains and horse bean, and the oil will be will be pressed from the rapeseed. The complete diets will be analysed for major nutrient content and value, and the diets will be freezer-stored until use. However, analyses of bioactive metabolites and minerals will be performed in accordance to WP3.

Task 2: The screening of the effects of dietary treatments with regard to health issues will be performed using a rat model. The type of rats will be chosen in order to ensure a sensitive animal model, which is required to catch all eventual effects of dietary treatments on health responses. It is suggested that the rat type will be the GKmodel. According to the statistical proposal (supplement no. xx) the experiment will be planned with five rats per dietary treatment. The rats will be fed from weaning until the end of the experiment. The experiment will last approximately 24 weeks, in order to test the effects of long-term consumption of diets on rats, which are full-grown. The following analyses are planned: The health measurements involve analyses on the rats while living (performance, clinical evaluation, physical activity) and post mortem: A macroscopic analysis of the organs and a visual determination of the animals will be determined in order to get a clinically evaluation of the health status. Blood and tissue samples will be obtained and analysed for biomarkers related to health (in accordance to the analyses performed during the project of FØJOII), such as lipid-profile (lipid classes and fatty acids), vitamins (E, A and β-carotene), selected antioxidants, and general nutritional status.

Task 3: As the immunological defence system is imprinted during the early life span, it is planned to investigate parameters of the humoral- and cell-mediated immune-system in rats continuously throughout the experiment starting at weaning, in order to obtain profiles of the immunological parameters during the lifespan. The rats will be offered the dietary treatments from weaning, and a control group (which will be fed standard rat chow) will also be included. The rats will after a period of consumption of the experimental feed be challenged in order to provoke the immune system. The choice of immunologial parameters to be analysed in the challenged rats will depend on the challenge. The literature will be thoroughly studied with respect to substances, nutrients etc. which have shown to influence the immune system, and which differ from one diet to another (between cultivation systems) in the present study. Planned parameters are representatives of the innate and adaptive immune system, and the humoral as well as the cell-mediated immune system. The department masters a broad spectre of these categories.

Task 4: Finally, selected sub-diets will be tested in a preference test with out-bred rats. The rats (Wistar) will be kept individually in a cage, and is allowed to choose among different diets, and the consumption of each diet will be measured daily. The method is detailed described in a recent paper (Yong et al., 2005), in which it was shown that rats were able to differentiate between diets of different cultivations systems, but that the dietary treatment of the mother rats influenced the conclusion. The preference test of the present study will be designed when the knowledge is available regarding the nutritional characterization of the foods and the general food quality is available in order to test eventual taste differences, which may appear according to the given cultivation systems.

Statistics:
Statistical tests will be applied in order to analyse the effect of cultivation system on rat health status in rats. After the first year of testing the most important response variables will be analysed using linear mixed models or generalised linear mixed models. The type of method will depend on the response variable. After two years testing the data from both years will be analysed together using the same type of models in order to evaluate to which extent the effects are consistent from year to year and to point out the effects of cultivation systems. This work is undertaken in close cooperation with DJF-GBI.

Deliverables:

D4.1 Differences among cultivation systems with respect to the development of the immune function (scientific manuscript with peer-review)

D4.2 The effect of cultivation systems with respect to biomarkers of health, antioxidant and nutritional status, and physical activity (scientific manuscript with peer-review)

D4.3 Preference test between diets of different cultivation systems (scientific manuscript with peer-review)

D4.4: Ph.D.–thesis with the suggested title: "Influence of organic food on health using the rat as a model for humans"

Milestones:

M4.1 Complete diets of ingredients from CROPSYS of cultivation year 1 are prepared

M4.2 Analytical methods regarding the development of the immune function of rats are available

M4.3 Rat experiments including chemical analyses of cultivation year 1 is performed

M4.4 Complete diets of ingredients from CROPSYS of cultivation year 2 are prepared

M4.5 Rat experiments including chemical analyses of cultivation year 2 is performed

M4.5 Rat experiment of cultivation year 2 is performed

M4.6 Overall data-analyses performed

Description of work packages (this page should be copied for each WP**)**

WP No.:	5 – Human bioavailability

	Start date or starting event: 05-2007								
Partner id.	KVL-IHE	DFVF-K1	DFVF-K2	KVL-PSSL	DJF-GBI				
Person-months per participant	50	14	10	6	1				
Total PM:	**81**								

(Please give Institution or Department as partner id.)

Objectives:
The primary aim of this study is to assess the bioavailability of bioactive compounds from different organic production systems in humans. More specifically the aims are:

1. To assess the bioavailability of Fe, Zn, and Se, from different organic production systems in humans.
2. To assess the bioavailability of selected bioactive secondary metabolites and vitamins (flavonoids, carotenoids, vitamin C, and E) from different organic production systems in humans.
3. To investigate the effects of interaction between trace elements and bioactive metabolites on absorption in humans from the different production systems.

Description of work:
Two human cross-over intervention studies will be performed in two different production years. In each study 15-18 healthy adult volunteers will be allocated to one of six diets for three times two weeks At day 7 in each period the volunteers will receive a diet labelled with ^{74}Se, ^{67}Zn and ^{54}Fe.
For the trace element investigations samples of diet, blood, urine and faeces will be collected at baseline. Furthermore, urine, and faecal samples will be collected from day 7 – 12 and blood samples will also be collected at the end of the intervention period. For investigations of bioactive metabolites and vitamins samples of diet and urine/plasma will be collected at baseline and urine/plasma also at the end of the intervention period. Similarly samples of diet and blood will be collected at baseline for investigations of vitamins and blood also at the end of the period.

Inclusion criteria
Healthy adults aged 18-45 years
BMI 20-30 kg/m^2
Non-vegetarian

Exclusion criteria
Physical activity > 10 h/week
Intake of dietary supplements
Regular use of drugs
Chronic diseases
Smoking
Excessive intake of alcohol

Ethics
Ethical approval will be obtained from the Local Research Ethical Committee of Copenhagen and Frederiksberg prior to recruitment of volunteers.

Advertisement and recruitment
Volunteers will be recruited by advertisement at local universities, colleges, and local newspapers. A standard subject information sheet will be produced as part of the ethical submission. A screening instrument will be administered over the phone and provided subjects meet the criteria will be booked in to a personal interview.

Provided the subjects meet the criteria outlined above they will be recruited to the study. Subjects will have the study explained to them again in greater detail and will sign a written consent form to participate in the study and to provide blood, urine and faecal samples.

Each subject will be provided with:
- a detailed subject information sheet
- a study diary in which all deviations from "normal" should be noted (illness, heavy exercise, use of painkillers etc.)
- a detailed water intake sheet
- a microwave oven if needed
- utensils for preparation and intake of food, hot beverages etc

Stratification and randomisation
Six diets from 3 systems in two locations in two repetitions will be produced according to the following plan:
A1 and A2) Diet based on products grown with green manure, B1 and B2) diet based on products grown on animal manure And C1 and C2) Conventional grown products.
Diets will be based on bread products and vegetables from the different production systems. All other ingredients in the diets will be kept at a minimum, but where needed, will be purchased from shops specialising in organic products for the organic diets and from supermarkets for the conventional diets.

All diets will be produced in the metabolic kitchen at Department of Human Nutrition and frozen until needed. During the intervention, lunch will be served at the department while breakfast; in-between snack and dinner will be packed and distributed each afternoon for consumption at home. The volunteers will be allowed free access to mineral water, as well as coffee produced with mineral water, which will be provided by the Department. To avoid contamination during intervention all utensils for cooking, as well as a microwave oven will be provided by the Department. Furthermore the volunteers will be instructed only to use the plastic forks, knives and plates provided by the Department., and only to heat food in the microwave oven and to prepare coffee with mineral water heated in glassware in the microwave oven.

Compliance
During the intervention lunch will be served at the Department of Human Nutrition under supervision. All other meals will be distributed for consumption at home. Subjects will be asked to return all food not eaten. This will be weighed and subtracted the subjects' dietary intake. The concentration of trace elements will be measured in urine and faeces and adjusted according to PABA concentration and numbers of faecal markers. Likewise the concentration of bioactive metabolites in urine will be adjusted.

PABA
At the days of urine collection, subjects will be asked to take one tablet of 80 mg para-amino benzoic acid (PABA) three times per day at their main meals. Urine will be collected in 2.5 L urine collection jars and will be aliquoted according to analyses and stored at -20°C or at –80°C as appropriate.

Sample collection and preparation
All samples for trace element analyses (blood, urine, faeces and double portions of the diet) will be collected in acid washed containers and trace element free tubes. Faecal and food samples will be freeze-dried and microwave digested before analysis. Blood and urine will be prepared according to further analysis and stored at ÷20 °C or ÷80 °C until analysis. Samples for analysis of other compounds (double portions of diet, blood, and urine) will be collected in tight containers, stored under nitrogen, ÷20 ºC or ÷80 ºC until further analysis.
Trace elements in food, urine, faeces, and blood will be determined by ICP-MS. The time resolved enrichment by ^{74}Se, ^{67}Zn and ^{54}Fe will be determined in blood and urine against an appropriate reference isotope by ICP-MS.
The bioactive metabolites (flavonoids, carotenoids, falcarinol, vitamin C and E) in diets, and in urine or plasma will be determined by LC and LC-MS investigations.

Inositol-phosphates (phytates) in cereals and beans ranging from IP1 to IP6 will be analysed by metal dye detection (MDD) HPLC according to standard protocols operated at KVL-PSSL.
All diet samples will be analysed for the selected chemical components relative to the dry matter content.

Statistics
The work will be related to trials carried out for testing the effect of cultivation system on human bioavailability. After the first year of testing the most important response variables will be analysed using linear mixed models or generalised linear mixed models. The type of method will depend on the response variable. After two years testing the data from both years will be analysed together using the same type of models in order to evaluate to which extent the effects are consistent from year to year and to point out the effects of cultivation systems. This work is undertaken in close cooperation with DJF-GBI.

Univariate mixed model analyses of covariance (ANCOVA) will be performed with absorption of trace elements and bioactive metabolites in the end of each period as dependent variables. Method and year of cultivation, order and period as well as method×year and method×period interactions will be evaluated as independent fixed variables and subject and location as independent random variables. Baseline absorptions will be evaluated as covariates.

Multivariate statistics will be applied to resolve the latent data structure and to examine the linkage between the chemical composition of soil and plant data with the human bioavailability data. Data will be examined according to the principles outlined in WP1 and will include PCA, SIMCA and PLS analysis.

Deliverables:

D5.1	Presentation of results at international conference
D5.2	Draft scientific publication "Bioavailability of micronutrients from different organic production systems in humans"
D5.3	Draft scientific publication " Bioavailability of bioactive metabolites from different organic production systems in humans"
D5.4	Draft scientific publication "Interaction between micronutrients and bioactive metabolites from different organic production systems in humans"
D5.5	Ph.D thesis

Milestones:

M5.1	Ethical approval obtained
M5.2	Production of diets for the intervention study, year 1, including the stable isotope labelled diet
M5.3	Recruitment of study population for the 1. year intervention study
M5.4	Diet (2007) analysed for Fe, Zn, Se and S
M5.5	Diet (2007) analysed for bioactive metabolites and vitamins
M5.6	Diet (2007) analysed for phytates
M5.7	Completion of dietary intervention study year 1
M5.8	Collection and distribution of human samples from 1.year intervention study
M5.9	Analyses of human samples from 1. year intervention study
M5.10	Production of diets for the intervention study, year 2
M5.11	Recruitment of study population for the 2. year intervention study
M5.12	Urine, plasma and faeces (2007 harvest) analysed for Fe, Zn and Se and enriched stable isotopes
M5.13	Urine and plasma (2007 harvest) analysed for bioactive metabolites and vitamins
M5.14	Diet (2008) analysed for Fe, Zn, Se and S
M5.15	Diet (2008) analysed for bioactive metabolites and vitamins
M5.16	Diet (2008) analysed for phytates
M5.17	Dietary intervention study year 2 completed
M5.18	Collection and distribution of human samples from 2.year intervention study
M5.19	Analyses of human samples from 2. year intervention study
M5.20	Estimation of absorption of trace elements
M5.21	Urine, plasma and faeces (2008 harvest) analysed for Fe, Zn and Se and enriched stable isotopes
M5.22	Urine and plasma (2008 harvest) analysed for bioactive metabolites and vitamins
M5.23	Estimation of absorption of bioactive metabolites
M5.24	Multivariate data analysis of data

A.3 Project resources and budget overview

(Short description, max. 1 page, of partners, key competences and management of the project. Overview of budget according to tables given below)

Key competences

All senior project partners are experienced researchers that have international networks (see attached CVs) to be used as appropriate for the benefit of the project.

KVL-PSSL:

The Plant and Soil Science Laboratory, Department of Agricultural Sciences, KVL has over the past years established extensive expertise in the functional relationships between soil nutrient bioavailability, plant productivity and plant quality. The laboratory has developed state-of-the-art techniques for elemental profiling and analysis of metal speciation in plants by HPLC-ICP-MS with octopole reaction cell (www.chime.kvl.dk). In addition, facilities for assessment of soil nutrient bioavailability and for analysis of plant metabolites such as amino acids, organic acids, anti-oxidants etc. have been established. The laboratory has experience in running field experiments with detailed monitoring of plant performance, soil and environmental parameters. The researchers involved in the present proposal have over the last 5 years received a large number of project grants from Danish research programs such High-value crops, Agriculture in a resource-holistic perspective, Harvest-Plus, STP-initiative on zinc etc. as well as EU-projects dealing with: plant nitrogen utilization (SUSTAIN), nitrogen deposition in terrestrial ecosystems (EXAMINE and GRAMINAE), metabolomic technology applications for plants, health and outreach (META-PHOR), Public health impacts of Cd exposure (PHIME). Previously, the laboratory has participated in FØJO projects on nitrogen fixation in clover-grass and has been responsible for three completed Ph.D. projects in organic agriculture and food systems.

KVL-IHE:

Department of Human Nutrition, Centre for Advanced Food Studies will be responsible for the human intervention studies. Dr. Bügel has more than 10 years experience in human intervention studies and the special concerns that have to be taken when dealing with micronutrients. The Department has kitchen facilities and dining rooms allowing for very large human dietary studies, as well as special rooms for blood collection and handling of both blood samples and urine and faeces samples. The laboratory is also well equipped for most routine clinical analysis as well as special research analysis.

DFVF-K:

Department of Food Chemistry has since many years conducted analyses and research on vitamins, minerals and mineral speciation, as well as secondary metabolites in foods and biological samples. For this purpose advanced coupled analytical techniques, based on mass spectrometry, like LC-MS and LC-ICPMS were used to produce analytical data, which were relevant for nutritional assessments. More specifically, we have evaluated the bioavailability of nutrients via the use of enriched stable isotopes, and have studies losses of compounds occurring during processing. Furthermore, the Department has a strong expertise in analyses of chemical contaminants in foods and biological samples.

DJF:

The Department of Animal Health, Welfare and Nutrition will be responsible for the rat studies. The scientists and technical personal involved in this workpackage have many years of experiences in performing clinical studies with rats, both with regard to the bioavailability of nutrients and to the influence of dietary treatment on the health and well-being of rats. The department houses several well-quipped laboratories for analyses of major- and minor nutrients, and advanced analytical techniques for the study of nutritional and antioxidant status, especially analytical competence regarding vitamins, fatty acids and lipids, antioxidants, metabolites etc. The department also masters a broad spectre of immunological responses, e.g. representatives of the innate and adaptive immune system, and the humoral as well as the cell-mediated immune system. In addition, the department has experimental facilities for measuring the physical activity and metabolism of energy and nutrients. The scientists involved in workpackage on the rat studies have several years of research experience within the area of nutrition, immunology and analytical chemistry.

Project coordination and management

The project will be managed by a Project Steering Committee (PSC) consisting of the leaders of the WP's and chaired by the project coordinator.

PSC meetings will be held at least twice a year. At the important first meeting the work plan in general and its time plan, the tasks, and the organization of the interaction between tasks will be reviewed and finalised. Especially the tasks for the first 12 months will be penetrated in depth. The latest version of available methods will be presented, and details in method selection will be decided. If necessary, a written agreement on patent issues and related matters will be worked out and included in a consortia agreement.

At the intermediary PSC meetings progress reports on each task will be presented. The work and interactions of the coming period will be discussed in detail, and any adjustments of the initial plan will be decided. Any possible emerging scientific and technical obstacles will also be discussed and the work plan will be adjusted accordingly. At these meetings also the progress reports to be delivered to DARCOF III will be discussed. The status of planned publications will be reviewed and an updated publication plan will be decided.

A research fellow with a degree in agronomy will be hired for the detailed planning, management and supervision of the field experiments incl. coordination and sampling of the mature crops, sample pretreatment, sample distribution to project partners, data analysis and reporting. The field trials will be undertaken by trained staff at the involved experimental stations.

Table for scientific person month allocated on WP's and partners

Partner / WP	1 KVL-PSSL	2 DFVF-K1	3 DFVF-K2	4 DJF-AWH	5 DJF-GBI	6 KVL-IHE	Total
WP1	22	0	0	0	0	0	22
WP2	12	15	13	0	0	0	40
WP3	12	14	19	0	0	3	48
WP4	0	0	0	49	1	0	50
WP5	6	10	8	0	1	21	46
Total	52	39	40	49	2	24	**206**

Table for breakdown of total budget on partners and different cost categories (DKK)

Participating institution	Responsi-ble scientist	Salaries		Equip-ment	Operational expenses	Total budget DKK
		Acade-mic	Techn. adm.			
KVL-PSSL	Søren Husted	1688	0	0	1283	3221
KVL-IHE	Susanne Bugel	700	660	0	473	2199
DFVF-K1	Pia Knuth-sen	1224	274	0	395	2272
DFVF-K2	Erik H. Larsen	1281	180	0	370	2197
DJF-AWH	Charlotte Lauridsen	1692	577	0	1033	3962
DJF-GBI	Kristian Kristensen	150	0	0	0	150
DJF-Foulumgård	Gunnar Mikkelsen	0	0	0		110.5
DJF-Jyndevad	Gunnar Mikkelsen	0	0	0		110.5
DJF-Flakkebjerg	Ilse Ras-mussen	0	0	0		110.5
DJFÅrslev	Kristian T. Kristensen	0	0	0		54
Total						**14.387**

***Note. Overhead is not included in salaries and operational expenses listed above. All specific details are available on attached budget breakdown table (Excel-file).*

A.4. Dissemination of scientific results

Dissemination

For dissemination of results Bakkegaarden-BioTrace will use the following mechanisms: scientific publications; targeting of important results directly to end-users, such as public and farmers (largely through advisers and national farmers organisations, but also directly through the farming press). In addition, reports of the results and their implications will be sent to policy makers and press. The results will be presented at various international and national congresses in organic farming, and at the SOAR Summer Schools. There will be input to educational courses, training courses and eLearning initiatives aimed at doctoral and postdoctoral training. A homepage presenting the project and its progress will be established including links to databases containing lists of recommended crop rotations, soil treatments and information on element profiles and bioavailability.

Education of researchers

The project will require employment of Ph.D. students for KVL-PSSL, DFVF-K, KVL-IHE and DJF. These students will be enrolled in the Research School for Organic Agriculture (SOAR) or other relevant research schools and benefit from a network of researchers within the project where frequent exchange and discussions of the results will be highlighted.

A.5. Scientific collaborations

All partners have an extensive international network of collaborative partners which will be included when appropriate. In the DARCOF III programme we will use our partnership in the international and interdisciplinary Harvest-Plus research programme (www.harvestplus.org) aiming at reducing micronutrient malnutrition by harnessing the powers of agriculture and nutrition research to breed nutrient dense staple foods. Also we are partners of EU-projects dealing with plant nitrogen utilization (SUSTAIN) and nitrogen deposition in terrestrial ecosystems (EXAMINE and GRAMINAE), metabolomic technology applications projects (META-PHOR), Public health impacts of Cd exposure (PHIME). We have also established contact to The Food Safety and Quality Unit under the European Commission, where Dr. Alain Maquet is heading a 3-year programme on multi-elemental traceability of organic and conventional plant products. We have agreed on exchanging students and expertise especially on analytical issues and multivariate data analysis methodology. The European Virtual Institute for Speciation Analysis (EVISA) at www.speciation.net will be used as an active platform for exchange of results and attraction of researchers for guest visits in the area of the proposal.

The Department of Animal Health, Welfare and Nutrition (DIAS) has been responsible for two work-packages in the project "Organic Food and Health – a Multigeneration Animal experiment", which was financially supported by DARCOFII. Collaboration with previous partners of this project will continue. In addition, close collaboration and coordination will be held to the projects "VEGQURE" and "CROPSYS" under DARCOFIII.

Collaboration with previous partners from DARCOF II will continue.

A.6 Other issues

Ethics

The local ethical committees will be applied for approval of the human intervention studies. All subjects are volunteers and are carefully informed both written and orally about the studies. They have to give written concept to participate.

The rat experiments will comply the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study.

The basic ethics and national legislation for organic farming in Denmark will be followed in all aspects of the project (www.plantedir.dk). All the planned activities are at present in full accordance with the current national legislation for organic farming in Denmark and during the running management and coordination of the project full attention will be given to this aspect.

A.7 References

Arthur 2003, Proc. Nutr. Soc, 393-397

Bergès et al. 2004, Carcinogesis, 25, 1953-1959

Brandt et al. (2001). HEALFO Conference, Santa Maria Imbaro and Lanciano - Italy, 13-15 June 2001.

Carpenter and Mahoney 1992 Crit. Rev. Food Sci. 31, 333-367

Day et al. 1998, FEBS Lett, 436, 71-75

Finamore et al. (2004). J. Agric. Food Chem. 52, 7425-7431.

Fox et al. 2003 ACS Symp. Ser. 835, 339-354

Gee et al 2000, J. Nutr. 130, 2765-2771

Gibson et al. 1997 Plant Foods Human Nutr. 51, 1-16

Graham et al. 2000 Asia Pacif. J. Clin. Nutr. 9, S91-S96.

Graham et al. 2000 Asia Pacif. J. Clin. Nutr. 9, S91-S96.

Grinder-Pedersen et al. 2003. J Agric Food Chem.51, 5671-6

Gu et al. 2003. J. Agric. Food Chem. 51, 7513-7521

Gundersen et al. 2000. J. Agric. Food Chem. 48, 6094-102

Hansen et al. 2003. J Sci. Food Agric. 83, 10010-1017

Hu 2003, Am. J. Clin. Nutr. 78 (3. suppl.), 544S-551 S.
Husted et al. 2004. Anal. Bioanal. Chem, 378, 171-182
Julshamn et al. 1978 Fisk. Dir. Skr. Ser. Ern. 4 137-144
Kidmose et al 2001, J Sci Food Agric, 81, 918-923
Knuthsen et al, submitted, J Food Comp Anal
Kopsell and Randle 2001 J. Am. Hort. Sci. 126, 329-335
Larsen et al. 2002, Food Addit. Contamin., 19, 33-46
Larsen et al. 2003 J. Anal. At. Spec. 18, 310-6.
Lauridsen et al. (2005). Submitted to British Journal of Nutrition.
Leth et al. 2001. Fødevaredirektoratet, Fødevarerapport 2001:17,
Levnedsmiddelstyrelsen 1995. Danskernes kostvaner 1995. Hovedresultater. Publikation 235, maj 1996.
Lyhne Andersen et al. (1996) Levnedsmiddelstyrelsen Publ. 235
Lyons et al. 2003 Nutr. Res. Rev. 26, 25-133
Mann S (2003) Food Policy 28, 459-469.
Nordic Nutrition Recommendations, 2004, 4th edition, Nord 2004:13, Nordic Council of Ministers
O'Doherty Jensen et al. (2001). Danish Ministry of Food, Agriculture and Fisheries, Copenhagen 10-11 May 2001, 172-177.
Prasad 1998 J. Trace Elem. Exp. Med. 11, 63-87
Prasad 1996 J Am. Coll. Nutr. 15, 113-120
Rayman 2002 Proc. Nutr. Soc., 203-215
Rayman et al. 2003 Am. J. Obstet. Gynecol. 189, 1343-1349
Ryan et al. 2004 J. Sci. Fd. Agric. 84, 207-216
Samsøe-Petersen et al 2002 Environ. Sci. Technol. 36, 3057-3063
Sandstrom et al.2000 J. Nutr. 130, 594-9
Schifferstein et al. (1998) Food Qual Pref 9, 119-133.
Sloth et al. 2005 J. Anal. At. Spec. In press.
Stavric 1994. Clin. Biochem.27, 319-32
Tapiero et al. 2003, Biomed. Pharmacother., 134-144
Thorup-Kristensen 2004, In: Olesen et al (Eds). DARCOF Report no. 1. pp. 133-140
Wei et al. 2004 Am. J. Clin. Nutr. 79, 80-85
Welch and Graham 1999 Field Crop Res. 60, 1-10
Welch and Graham 2004 J. Exp. Bot 55, 353-364
Williams CM (2002). Nutritional quality of organic food: shades of grey or shades of green? Proc. Nutr. Soc. 61, 19-24.
Woese et al. (1997) J Sci Food Agric 74, 281-293.
Yong et al. (2005). mitted to the J. Food Sci. Chem.

Curriculum Vitae – Pia Knuthsen

Personal data

Born: 14 August 1949
Address: Ledreborg Allé 8, DK-2820 Gentofte
Tlf. + 45 39 69 08 81
Civil status: Married to Jens Juul Rasmussen
Three children (born in 1984, 1987, 1992)

Education

M.Sc. from the Danish Technical University, 1975

Positions

1975-1977 Chemist, Danish Institute for Food and Veterinary Research (temporary position)
1977-1990 Chemist, Danish Institute for Food and Veterinary Research
1990-2003 Senior Research Chemist, Danish Institute for Food and Veterinary Research. Substitute for head of department, temporary (2003 January-December).
2004- Senior Scientist, Danish Institute for Food and Veterinary Research

Research areas

Current research interests are focused on nutrients and bioactive secondary metabolites, and contents in foodstuffs and biological samples. Recent work includes research within bioactive metabolites like flavonoids and other polyphenols, and glycoalkaloids. Analytical methods are developed, e.g. based on HPLC with diodearray and MS or MS-MS detection, for making surveys of contents in food, feed or biological samples. Projects include determination of contents of selected substances in foods, and their variation with growing conditions, production of foods and cooking. Our projects often form part of larger cooperative research, national or international, e.g. EU projects.
Food content results are often used for calculations of human intakes in coorperation with the nutritional department at our institute. Such results also form part of the basis of the Danish food composition tables.

Teaching etc

- Lecturing at course in Natural antioxidants at the Royal Veterinary and Agricultural University (KVL), 1999-present.
- Supervisor of master students. - External examiner for master and bachelor thesis students, and at PhD courses at KVL, 1990-present.

Other matters

Danish representative and member of the management committee of Cost Action 926, Impact of new technologies on the health benefits and safety of bioactive plant compounds (Feb. 2004-2008).

Publications (selected peer reviewed papers)

Metzdorff SB, Kok, EJ, Knuthsen P, Pedersen J (2006). Evaluation of a non-targeted "omic" approach in the safety assessment of genetically modified plants. Accepted for publ. In Plant Biology.

Bergquist SÅM, Gertsson UE, Knuthsen P, Olsson ME (2005). Flavonoids in baby spinach (*Spinacia oleracea* L): Changes during plant growth and storage. J Agric Food Chem, 53, 9459-9464

Krogholm KS, Haraldsdóttir J, Knuthsen P, SE Rasmussen SE (2004). Urinary total flavonoid excretion but not 4-pyridoxic acid or potassium can be used as a biomarker for the intake of fruits and vegetables. J Nutr 134, 2, 445-451.

Knuthsen P, Madsen JS, Hansen KS, Leth T. Monitoring nutrients in fruits and vegetables. J Food Comp Anal (submitted).

Breinholt VM, Nielsen SE, Knuthsen P, Lauridsen ST, Daneshvar B, Sørensen A (2003). Effects of commonly consumed fruit juices and carbohydrates on redox status and anticancer biomarkers in female rats. Nutrition and Cancer 45, 1, 46-52.

Kidmose U, Knuthsen P, Edelenbos M, Justesen U, Hegelund E (2001). Carotenoids and flavonoids in organically grown spinach (*Spinacea oleraca* L) genotypes after deep frozen storage. J Sci Food Agr 81: 918-923.

Curriculum vitae for Charlotte Lauridsen

Born: January 26, 1969 in Gentofte, Denmark

Educations: 1998: Ph.D., Food Science Chemistry, Royal Veterinary and Agricultural University
1994: M.Sc., Animal nutrition and physiology, Royal Veterinary and Agricultural University

Employments:

1994-1995:	Research Training Fellow, Department of Nutrition, University College Cork, Ireland
1995-1998:	Ph.D.-student, Department of Product Quality, Danish Institute of Agricultural Sciences (DIAS), Denmark
1998-2001:	Scientist, Dept. of Animal Nutrition and Physiology, DIAS.
2001-2005:	Senior scientist, Department of Animal Nutrition and Physiology, DIAS, Denmark.
2005-	Senior scientist, Dept. of Animal Health, Welfare and Nutrition, DIAS, Denmark.

Leave of absence: 28/3-1/11-2000: Research Associate, Linus Pauling Institute, Oregon State University, Oregon, USA.

Supplementary education

1) *Projektorganisation og ledelse*, Handelshøjskolen, Exam obtained June 2005
2) *Kursus i Mixed Models*, Forskningscenter Foulum, 2001-2002.
3) *Course in lipid biochemistry and metabolism related to biotechnology – An introduction to advanced analytical methods and lipid biochemistry*. Technical University of Denmark, 2000.
4) *Projektlederkursus (modul I + II)*. Forskningscenter Foulum, 1999.

Research profile and other competences: My research activities concern research regarding nutrition and physiology of animal husbandry, with major focus on the importance of vitamins (vitamin E, A, C og D), fat, fatty acids, natural and synthetic antioxidants and selected minerals for monogastric animals (swine, poultry and rats) liveability, health, reproduction, nutrient utilisation, and product quality. The research involves basic and applied physiological investigations with special emphasis on the absorption and metabolism of fat and fat-soluble vitamins. I have ongoing activities regarding lecturing and supervision of students, primarily from the Royal Veterinary and Agricultural University, and both Danish students and students from the third-world**.** Furthermore, I have experience with commercialisation of research results, and I have several years of practical experience with project-leading and coordination (e.g. projects financed by the Danish Agricultural and Veterinary Research Council, Ministry funding, and private industries), , among them leading of sub-projects of the project "Organic Food and Health – a multigeneration experiment" (DARCOF II). Further information:
http://www.agrsci.dk/afdelinger/forskningsafdelinger/hef/medarbejdere/cla

I have in total 101 publications (1/5 2006), of which 26 have been published in international journals with peer-review. Following key-publication should be mentioned:

Lauridsen, C., Engel, H., Craig, A.M. & Traber, M.G., 2002. Relative bioavailability of dietary RRR- and all-rac-alpha-tocopheryl acetates in swine assessed using deuterium-labeled vitamin E. Journal of Animal Science 80, 702-707.

Lauridsen, C., Engel. H., Jensen, S.K., Craig, A.M. & Traber, M.G., 2002. Lactating sows and suckling piglets preferentially incorporate RRR- over All-rac-alfa-Tocopherol into milk, plasma and tissues. Journal of Nutrition 132, 1258-1264.

Lauridsen, C. and Danielsen, V. 2004. Lactational dietary fat levels and sources influence milk composition and performance of sows and their progeny. Livestock Prod. Sci., 91, 95-105.

Lauridsen, C. and Jensen, S.K. 2005. Influence of supplementation of vitamin E preweaning, and vitamin C postweaning on vitamin E- and immune responses of piglets. Journal of Animal Science, in press.

Lauridsen, C., Yong, C., Halekoh, U., Bügel, S.H., Brandt, K., Christesen, L.P., and Jørgensen, H. 2005. Rats show differences in health when eating diets based n ingredients produced with three different cultivation strategies. Submitted to British Journal of Nutrition.

Other publications: http://web.agrsci.dk/ArsPublikationer/PubliResult.asp

Role in project: Project-leader (responsibility and active participation) in the work-package involving the influence of cultivation systems regarding health of rats.

Curriculum vitae, Søren Husted

DATE OF BIRTH	April 3, 1963
MARRITAL STATUS	Married to M.Sc. Ph.D. Anne Skriver. Two children (Laura and Markus; 13 and 9 years old).
ADRESS	Plant and Soil Science Laboratory, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University (KVL) DK-1871 Frederiksberg C, Copenhagen, Denmark Tel: +4535283498, Fax: +4535283460 E-mail: shu@kvl.dk ; homepage: http://www.agsci.kvl.dk/~sohuj5/shu2001/index.htm
ACADEMIC DEGREES	1991: M.Sc in Chemistry at The Royal Veterinary and Agricultural University (KVL) 1997: Ph.D. in Plant Nutritional Physiology & Analytical Chemistry (KVL)
EMPLOYMENT	From 1991 to 1993: Research Associate at the Danish Institute of Agricultural Sciences From 1993 to 1997: Ph.D. student at the Plant Nutrition Laboratory (KVL) From 1997 to 2001: Assistant Professor at the Plant Nutrition Laboratory (KVL) From March 2000 to October 2000: Visiting Scientist at University of Western Australia Since April 2001: Associate Professor at the Plant Nutrition Laboratory (KVL)
RESEARCH PROFILE	Most of my research is focusing on the the functional role of the essentiel elements in plants and on the dynamics of trace elements in the rhizosphere. Currently, special attention is given to essential micronutrients in plants with special emphasis on manganese deficiency in Danish agriculture. I have expertise with plant cultivation in green houses, climate chambers and in the planning and evaluation of field experiments. I have gained expertise with the use of stable and radioactive isotopes and with multielemental and trace element analysis using ICP-OES, ICP-MS and IR-MS. I also have major expertise in using various chromatographic techniques such as HPLC, FPLC and IC with and without coupling to ICP-MS and ESI-MS. In recent years I have extensively used multivariate data analysis techniques to perform data mining of large multielemental data matrices. At present we have initiated new research projects on elemental speciation in the rhiszopshere (funded by the Danish Research Council) and in cereal grains (funded by the HarvestPlus programme) using LC-ICP-MS and LC-ESI-MS.
EDITORIAL	Reviewer international journals, including: *Journal of Analytical Atomic Spectroscopy (JAAS), Analytical and Bioanalytical Chemistry, Analytical Chemistry, Plant Physiology, Physiologia Plantarum, Annals of Botany, New Phytologist, Plant and Soil, Reviewer for the Norwegian Research Academy*
FUNDING	Funding is provided by The Agricultural and Veterinary Research Council; EU FP5, DANIDA/HarvestPlus, The Ministry of Food, Fisheries and Agriculture; The Carlsberg Fundation, The Cereral Breeding Foundation, Kemira and a number of other private research foundations.
TEACHING	Involved in teaching at undergraduate, graduate and postgraduate levels. Primary Lecturer in nutritional physiology at Bachelor level and in Applied Plant Nutrition and Analytical Chemistry at Master level. Since 1997, supervised numerous B.Sc, M. Sc. and Ph.D projects. Nominated with the Best Teacher of the Year Award "The Golden Bull" in 1999 and 2001 at KVL.
PATENTS & INNOVATION	Winner of the innovation price 2005 (Agro Business Park) with NutriNostica: Early diagnosis of manganese deficiency in cereals. Patent pending. Has in 2006 started a university based private company: NutriNostica Aps.
PUBLICATIONS	To this date 53 publications have been produced of which 40 have been published in anonymously refereed international journals. "Top-3 Best Paper Award 2004" for a paper in Analytical and Bioanalytical Chemistry 378: 171-182

Persson D, Hansen TH, Holm PE, Schjørring JK, Cakmak I, **Husted S** (2006) Multi-elemental speciation analysis of barley genotypes differeing in tolerance to Cd toxicity using LC-ICP-MS and ESI-TOF-MS. JAAS (submitted)

Tiryakioglu M, Eker S, Ozkutlu F, **Husted S**, Cakmak I (2006) Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. Journal for Trace Elements in Medicine and Biology (accepted)

Pedas P, Hebbern CA, Schjoerring JK, Holm PE, **Husted S** (2005) Differential capacity for high affinity manganese uptake contributes to differences between barley genotypes in tolerance to low manganese availability. Plant Physiology, 139, 1411-1420

Jakobsen MK, Poulsen LR, Schulz A, Fleurat-Lessard P, Møller A, **Husted S**, Schiøtt M, Amtmann A, Palmgren MG (2005) Pollen development and fertilization in Arabidopsis is dependent on the male gametogenesis impaired anthers gene encoding a Type V P-type ATPase, Genes and Development, 19, 2757-2769

Schjoerring JK, Maeck G, Nielsen KH, **Husted S**, Suzuki A, Driscoll S, Boldt R, Bauwe H (2005) Antisense reduction of serine hydroxymethyltransferase results in diurnal displacement of NH4+ assimilation in leaves of Solanum tuberosum L. The Plant Journal, 45, 71-82

Hebbern CA, Pedas P, Schjoerring JK, Knudsen L, **Husted S** (2005) Genotypic differences in manganese efficiency: field experiments with winter barley (Hordeum vulgare L.). Plant and Soil, 272, 233-244

Curriculum Vitae: Susanne Gjedsted Bügel, born Poulsen, born 25. July 1961

Address: **Department of Human Nutrition and Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C.**

Education:

1990: M. Sc. (Human nutrition), Odense University.
1996: Ph.D (Environmental Medicine), Odense University.

Employment:

2002- Associate Professor. Department of Human Nutrition, KVL
2004- 2005 Head of section (Preventive nutrition)
2005- Head of Education (MSc in Human Nutrition)

Research areas: Research areas include bioavailability of micronutrients and bioactive dietary components and prevention of lifestyle diseases. Has participated in a number of EU, FØTEK and FØJO financed projects i.e. DIETOX, (effect of changed fatty acid composition in pork and lard on risk marker for cardiovascular diseases in young men). FOODCUE, (effect of copper on cardiovascular risk factors in young women); OSTEODIET, (vitamin Ks effect on bone health in post-menopausal women) and, as coordinator for ISOHEART, (effect of isoflavones on risk markers for cardiovascular disease in postmenopausal women). The FØTEK financed projects has primarily focused on bioavailability of micronutrients from different diets and after different production processes.

Publication: In total 73 publications (01/05/2006) of which 20 have been published in international journals with peer review. The most recent:

1. **Bügel S**, Harper A, Rock E, O'Connor JM, Bonham MP, Strain JJ. Effect of copper supplementation on indices of copper status and certain cardiovascular disease risk markers in young healthy women. Br J Nutr 94, 231-6, 2005
2. Koebnick C, Reimann M, Carlsohn A, Korzen-Bohr S, **Bügel S**, Hallund J, Rossi L, Branca F, Hall W, Williams C, Zunft HJF, O'Doherty Jensen K. The Acceptability of Isoflavones as a Treatment of Menopausal Symptoms: a European Survey among Postmenopausal Women. Climacteric 8, 230-42, 2005
3. Bach Kristensen M, Hels O, Morberg C, Marving J, **Bügel S**, Tetens I. Pork meat increases iron absorption from a 5-day fully controlled diet when compared to a vegetarian diet with similar vitamin C and phytic acid content, Br J Nutr 94, 78-83, 2005
4. Hall WL, Vafeiadou K, Hallund J, **Bügel S**, Koebnick C, Reimann M, Ferrari M, Branca F, Talbot D, Dadd T, Nilsson M, Dahlman-Wright K, Gustafsson J-Å, Minihane A-M, Williams CM. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr 82, 1260-8, 2005
5. Kristensen MB, Hels O, Morberg CM, Marving J, **Bügel S**, Tetens I. Total zinc absorption in young women, but not fractional zinc absorption, differs between vegetarian and meat based diets with equal phytic acid content. Br J Nutr 94, 963-7, 2006
6. De Pascual-Teresa S, Hallund J, Talbot D, Schroot J, Williams CM, **Bügel S**, Cassidy E. Absorption of isoflavones in humans: effects of food matrix and processing. J Nutr Biochem 17, 257-64, 2006
7. Reimann M, Dierkes J, Carlsohn A, Talbot D, Ferrari M, Hallund J, Hall WL, Vafeiadou K, Huebner U, Branca F, **Bugel S**, Christine CM, Zunft H-J F, Koebnick C. Consumption of soy isoflavones does not affect plasma total homocysteine or asymmetric dimethylarginine concentrations in healthy postmenopausal women. J Nutr 136, 100–5, 2006
8. Hallund J, Ravn-Haren G, **Bügel S**, Tholstrup T, Tetens I. A lignan complex isolated from flaxseed does not affect plasma lipid concentrations or antioxidant capacity in healthy postmenopausal women. J Nutr 136, 112-6, 2006
9. Hallund J, **Bügel S**, Tholstrup T, Ferrari M, Talbot D, Hall WL, Reimann M; Williams CM, Wiinberg N. Soy isoflavone enriched cereal bars affect markers of endothelial function in postmenopausal women. Br J Nutr. In press.
10. **Bugel S**, Sørensen SD, Hels O, Kristensen M, Vermeer C, Jakobsen J, Flynn A, Mølgaard C, Cashman K. Effect of phylloquinone supplementation on biochemical markers of vitamin K status and bone turnover in postmenopausal women. Br J Nutr. In press.

CURRICULUM VITAE - KRISTIAN KRISTENSEN

Name and birth
Kristian Kristensen, born 27.4.1945

Scientific degrees
Cand. hort. Royal Veterinary and Agricultural University, Copenhagen, 1970
Ph.D. Department of Mathematics and Statistics, Royal Veterinary and Agricultural University, Copenhagen, 1980

Employment record
1970-76. Research assistant, Danish Institute of Plant and Soil Science (SP)
1976-79. Ph.D student. Department of Mathematics and Statistics, Royal Veterinary and Agricultural University, Copenhagen
1979-1987. Research assistant, SP
1987-1992. Temporary head of Department of Biometry and Informatics, SP
1992-1998. Scientist, SP/Danish Institute of Agricultural Sciences (DIAS)
1998- Senior scientist, DIAS.

Research areas
Many years experience in applied statistics. Especially analyses of data from crop and soil science using different kind of methods, such as linear and non-linear mixed model, generalised mixed models and other multivariate models. Participation in projects on setting up and evaluation of models for nitrogen leaching from agricultural fields (N-LES, N-LES_2 and N-LES_3). Great expertise in design of experiments.

Other activities
Participates in COST action 860, where he coordinates WP2 on biostatistics. Planned and coordinated workshops on DUS data in Kiew (2000), Mexico (2002) and Beijing (2004). Teaching on courses on statistical matters.

Publications
Published 28 papers in international scientific journals, 45 papers at conferences, 47 other papers and reports and more than 100 oral presentations.

Refereed publications 1st Januray 2004-2006 7th June 2006:

Hansen, E.M.; Christensen, B.T.; Jensen, L.S.; Kristensen, K.. 2004. Carbon sequestration in soil beneath long-term *Miscanthus* plantations as determined by ^{13}C abundance. Biomass and Bioenergy **26**, 97-105.

Yohalem, D.S. & Kristensen, K. 2004. Optimisation of timing and frequency of application of the antagonist *Ulocladium atrum* for management of grey mould in pot rose under high disease pressure. Biological Control **29**, 256-259.

Eriksen, J.; Askegaard, M. & Kristensen, K. 2004. Nitrate leaching from an organic dairy crop rotation: the effect of manure type, nitrogen input and improved crop rotation. Soil Use and Management 20, 48-54.

Kristensen, K.; Hansen, P.K. & Kristoffersen, P. 2004. Simulation of vegetation cover on sidewalks in Denmark. Pest Management Science **60**, 588-594.

Hansen, P.K.; Kristoffersen, P. & Kristensen, K. 2004. Strategies for non-chemical weed control on public paved areas in Denmark. Pest Management Science **60**, 600-604.

Gislum, R.; Boelt, B., Jensen; E.S., Wollenweber; B. & Kristensen, K., 2005. Temporal variation in nitrogen concentration of above ground perennial ryegrass applied different nitrogen fertiliser rates. Field Crops Research **91**, pp. 83-90.

Askegaard, M., Olesen, J. & Kristensen, K. 2005 Nitrate leaching from organic arable crop rotations: effects of location, manure and catch crop. Soil Use and Management **21**, 181-188.

Rabølle, M.; Spliid, N.H.; Kristensen, K. and Kudsk, P. 2006 Determination of Fungicide Residues in Field-Grown Strawberries following Different Fungicide strategies against Grey Mould (*Botrytis cinerea)* Journal of Agricultural and Food Chemistry. **54**, 900-908.

Rasmussen, I.A.; Askegaard, M.; Olesen, J.E.; Kristensen. K. 2006. Effects on weeds of management in newly converted organic crop rotations in Denmark. Agriculture, Ecosystems & Environment **113**, 184-195.

Curriculum vitae

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Born: November 8, 1950.

Job positions: 1977Analytical chemist (research associate) in the Danish Veterinary and Food Administration.

1993 Ph.D. degree awarded by the Royal Danish School of Pharmacy. Title of thesis: Arsenic speciation in food. Development of analytical methods and their application to biological samples and food.
1993-2004 Senior Research Chemist at the Danish Veterinary and Food Administration, Institute of Food Chemistry and Nutrition.

2004 Research professor in analytical chemistry at Danish Institute for Food and Veterinary Research

Job profile:

Team leader in the Trace Elements and Minerals Group. Research on trace elements in foods and human samples, particularly speciation research using HPLC-ICP-MS and –ESI-MS/MS. Research work on arsenic, selenium and iodine speciation. Research steering of internally and externally funded projects. Research-based advisory role for Danish Veterinary and Food Administration.

References (selected within the scope of the application):

Larsen, E.H., Lobinski, R., Burger-Meÿer, K., Hansen, M. Ruzik, R., Mazurowska, L., Have Rasmussen, P., Sloth, J.J., Scholten, O. and Kik, C., Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate, *Anal. Bioanal. Chem.*, In press.
Larsen, E.H., Hansen, M., Paulin, H., Moesgaard, S., Reid, M. and Rayman, M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemo-prevention studies *J. AOAC International,* 2004, **87**(1), 225-232.
Sloth, J.J., Larsen, E.H., Bügel, S. and Moesgaard, S., Determination of total selenium and ^{77}Se in isotopically enriched human samples by ICP-dynamic reaction cell-MS, *J. Anal. At. Spectrom.*, 2003, **18**, 317-322.
Larsen, E.H., Sloth, J.J., Hansen, M. and Moesgaard, S., Selenium isotope composition and speciation in intrinsically ^{77}Se-labelled yeast using gradient elution HPLC separations and detec tion by ICP-dynamic reaction cell-MS *J. Anal. At. Spectrom.*, 2003, **18**, 310-316.
Larsen, E.H., Hansen, M., Fan, T. and Vahl, M., Speciation of selenoamino acids, selenonium ions and inorganic selenium by ion exchange HPLC with mass spectrometric detection and its application to yeast and algae, *J. Anal. At. Spectrom*., 2001, **16**, 1403-1408.
Højbjerg, S., Sandström, B. and Larsen, E.H., Absorption and retention of selenium from shrimps in man, *J. Trace Elements Med. Biol.*, 2001, **14**, 198-204.
Sloth, J.J. and Larsen, E.H., The application of inductively coupled plasma-dynamic reaction cell-mass spectrometry for measurement of selenium isotopes, isotope ratios and chromatographic detection of seleno-amino acids, *J. Anal. At. Spectrom.,* 2000, **15**, 669-672.