

SKILLEBY LONG TERM FIELD TRIAL 1991-2010

Final report June 2011



The Biodynamic Research Institute

Skilleby long-term trial started in 1991 and still continuing



Experimental plan from 1991

Main plot	Treatments winter wheat
F1	Not composted manure 12.5 ton (0 from 1995)
F2	25 ton
F3	50 ton
K1	Composted manure 12.5 ton (0 from 1995)
K2	25 ton
K3	50 ton
Subplots +	BD preparation each plot each year
-	Without BD preparation

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Background

At the Biodynamic Research Institute in Järna, Sweden three sets of long-term field trials have been conducted since 1958. The basic aim was to develop biodynamic farming during Nordic conditions. The experiments started as an initiative within the Nordic Research circle for biodynamic agriculture, founded 1949 with members from the all Nordic Countries.

K-trial in Järna 1958 -1990

This trial period lasted for 32 years from 1958 to 1990. The main research question concerned the quality of agricultural food products under different farming and especially fertilizing conditions. Also the interaction and influence on soil fertility of different manuring techniques were studied¹⁻⁴. The difference between a cultivation that uses organic fertilizer compared to one that uses mineral fertilizer and where both achieves comparable yield-levels can according to the results from the K-trial be summarized as:

Soil

- higher enzyme-activity, soil respiration and occurrence of earthworms
- more deep going soil processes
- considerably higher nitrogen-mineralising capability
- better soil-fertility

Crop

- better storage efficiency and resistance against decomposition
- higher grade of maturity
- higher amount of leguminous plants in the clover/grass ley

The fully biodynamic treatment was characterised by a considerable higher amount of carbon in the soil, especially below plough depth. The biodynamic treatment also decreased the sensibility of the plant against potato blight. The biodynamic preparation tended to increase the yield during years when the common yield level was low and to decrease yields when the common yield level was high.

UJ –trial 1971 -1979

The results from the initial K-experiment formed the basis for one 6 and one 9-year trial, which were carried out by the Biodynamic Research Institute in collaboration with SLU (Swedish University of Agricultural Sciences) on two locations, in Ultuna near Uppsala and in Järna. The field trials had the same layout on both locations; four replications and all three crops in the three year crop rotation each year. In the trials Biodynamic cultivation with and without leys was compared with conventional cultivation with and without leys in three-year crop rotations. These trials resulted in the first doctoral dissertation within biodynamic cultivation in Sweden⁵ and a report from the 9-year trial in Järna⁶. Results from the two trials corresponded well with each other as well from the K experiment⁷. This means that the biodynamic treatment in comparison with the conventional had:

- 10 % less yield (summer wheat)
- Less storage losses (potato)
- Higher dry matter content (potato)
- Superior protein quality (potato)
- Higher content of vitamin C (potato)
- Better cooking quality and taste (potato)

Skilleby long term trial 1991-2011

When the K-experiment ended in 1990 field studies were established within Skilleby research farm. This made it possible to evaluate the consequences of different treatments within a given farm situation. The aim was to study how the farm's own manure could best be treated and used to promote soil fertility characteristics, the economic use of nutrients, the level of output (yield) and the nutrient quality of the products, while minimising negative impacts to the environment, all in the context of biodynamic agriculture ^{8,9}

Acknowledgments

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Introduction

The concept of food quality is diverse. It must be dealt with from many different angles and on many different levels. In this report we try to describe some of the aspects and questions in connection with food quality.

The golden thread that runs through this report can be formulated into questions like:

- Are there correlations between the fertility of the soil, the quality of the crop and the health effects of the food?
- What concepts are adequate to use in connection with these questions?
- What methods are suited to investigate if there are such correlations?

The basic properties of our food arise on farms, during specific situations. Most farmers try to maximise the yield and the quality of their crops. The specific system used on a farm is a consequence of the farmer's way of thinking. During the course of the project reported here two basic approaches have been discussed:

In the linear model the farming system is designed more or less like an assembly band. In the circular model the farming system is designed as a unit, a living organism.

The linear model tries to maximise the outcome of every crop by optimising the inputs used, by protecting the crop from predators and by rationalising the techniques used. The farmer's focus is concentrated on the specific crop. As an example the manure or fertiliser is used to satisfy the nutritional needs of the actual crop. Most inputs such as fertilisers and pesticides are imported from outside the farm.

The circular model has its main focus on the farm as such. It tries to build up a sustainable ecosystem by optimising the crop rotation, balancing fodder and animal production, encouraging the diversity of the wild flora and fauna. Manures are used as a way of improving the fertility of the soil. Most inputs arise from the farm itself and the food exported is a result of a surplus produced by the farm organism.

Between the extremes of these two approaches there are many different ways of designing a farm. Also organic farming can be described in terms of these two approaches.

Very little is known about the consequences of these approaches on soil fertility and food quality, even less about the consequences on human health.

The vitality concept

Many different concepts have been used when trying to describe the phenomenon discussed here. The most common is the "compound" concept. According to this, connections between farming system, soil fertility, food quality and human health are discussed in terms of the compounds involved. The compounds discussed have changed over time. It has been nitrogen, vitamins, minerals, anti-oxidants etc. Another angle to approach the topic is the concept of vitality. This concept can be summarized as follows: "*vitality is the ability of an organism to maintain itself under pressure from its environment*". It can be applied to different levels of food production

Farm vitality is thus the ability of a farm to maintain and develop itself and produce a surplus under different annual variations.

Soil vitality can be expressed as the ability to withstand drought or rain or the impact of heavy machinery and still offer good conditions for plant growth.

Crop vitality can be expressed as the plants resistance to decomposition and ability to withstand pests but also as the plants own ability to grow and develop, to reach a mature stage at a high yield level.

Consumers vitality can be expressed as the experience of well-being or as the ability to handle the problems faced in life, like poor health or personal crisis.

Methodological approaches

The concept of vitality is more suited to characterise than to define. This means that an understanding of the concept can be achieved by looking from different angles, by trying to describe connections between the results from different methods. From this it is clear that in order to characterise the concept of vitality many different methods and approaches must be used. These can be grouped in different ways:

Quantitative methods

The yield level and the weight, length or thickness of harvested crop

Chemical methods

Amount of different chemical compounds in soil and crops

Sensory/ morphological methods

The shape, taste, smell, warmth etc of the object analysed

Holistic methods

Picture forming methods such as biocrystallisation and paper chromatography, biophoton measurement, electrochemical methods a.o.

Reactions of living organisms or tissues

*The impact of the object on living cell cultures or living tissues
Intervention studies.*

In order to evaluate Skilleby long term field experiment methods from the first four groups have been used.

Biodynamic farming

The carrot, *Daucus carota* L

Cultivated carrots can be divided into two types. Eastern, asiatic, carrots have reddish purple or yellow roots, pubescent leaves and a tendency for early flowering. Western carrots have orange, yellow, red or white roots, less pubescent, green leaves and less tendency to bolt¹⁰.

The origin of the Eastern cultivated carrot is regarded to be in the Inner Asiatic Centre, mainly Afghanistan, and the origin of western cultivated carrot in the Asia Minor Centre, primarily Turkey¹¹. It exist little evidence of cultivating western carrots before the 10th century. Carrot

seeds of this type have been found in Switzerland and Germany, dating from 2000- 3000 BC. Probably at this time the seed was the plant part used¹². Purple, red and yellow carrots of the western type were cultivated in Iran in the 10th century and spread to China and Europe during the 13th century. The origin of the western orange type is not clear. The first appearance goes back to oils paintings from Holland during the 17th century. Written documentation of orange carrots first appears in 1721 with the description of 4 different orange carrot types. At approximately the same time the first white carrot was described in Holland also¹². A brief overview of the origins of cultivated carrot is given in table 1.

Table 1. History of cultivated carrot¹⁰.

Time	Location	Colour
Pre-900s	Afghanistan and vicinity	Purple and yellow
900s	Iran and northern Arabia	Purple and yellow
1000s	Syria and North Africa	Purple and yellow
1100s	Spain	Purple and yellow
1200-1300	Italy and China	Purple and yellow
1300s	France, Germany, The Netherlands	Purple and yellow
1400s	England	Purple and yellow
1600s	Japan	Purple and yellow
1600s	Northern Europe and North America	Orange and white
1700s	Japan	Orange

Botany

The edible carrot, *Daucus carota* var. *sativus* Hoffm., is part of the Apiaceae- family. Some of the representatives of this family and their common uses are listed in table 2.

Table 2. Representatives of the Apiaceae-family and their uses¹⁰.

English name	Swedish name	Botanical name	Uses	Plant portion used
Carrot	Morot	<i>Daucus carota</i>	F	R
Dill	Dill	<i>Anethum graveolens</i>	F	L, Fl, S
Parsnip	Palsternacka	<i>Pastinaca sativa</i>	F	R
Celery	Selleri	<i>Apium graveolens</i>	F	R
Parsley	Persilja	<i>Petroselinum crispum</i>	F	L, R
Caraway	Kummin	<i>Carum carvi</i>	F	S
Fennel	Fänkål	<i>Foeniculum vulgare</i>	F	S, R
Anise	Anis	<i>Pimpinella anisum</i>	F, M	S
Lovage	Libsticka	<i>Levisticum officinale</i>	F, M	L
Coriander	Koriander	<i>Coriandrum sativum</i>	F	L, S
Angelica	Kvanne	<i>Angelica archangelica</i>	M	L,R, S, St
Bishop's weed	Kirskål	<i>Aegopodium podagraria</i>	W (F)	L
Hemlock	Odört	<i>Conium maculatum</i>	M	

(F=food, M=medical, W=weed, Fl=flower, L=leaves, R= roots, S=seeds, St= stems)

Characteristic of the Apiaceae family is their compound umbel (umbrella-like) inflorescence. The separate flowers and umbels are often arranged in a well-coordinated inflorescence. The separate flowers are not so outstanding, they are often white or yellowish, and more seldom pale red or blue. Although the scent of the flowers often is weak, many species of the Apiaceae possesses a strong distinctive aroma. This aroma is due to essential oils, often produced in special oil ducts situated in the leaves, stem or roots. The composition of these

oils is unique for each species and is sometimes poisonous. In Apiaceae alternate compound leaves are common characteristic.

Anatomy

Soon after emergence the young carrot seedling show a clear difference between the taproot and the hypocotyl. The latter is, at first, thicker and bears no lateral roots. The upper part of the hypocotyl is terminated at the cotyledonary node. Here the bases of the cotyledons gradually merge with the hypocotyl.

Most of the storage root is comprised of phloem and xylem together with cambium sections gradually joining together in a cylinder. The anatomy of the carrot storage root is shown in figure 1 on the next page.

The shape of the storage root varies from round over conical to cylindrical. Depending on the pigment composition carrots can appear orange, yellow, red, purple or white. Shape and colour are mainly caused by genetic factors but can also be influenced by environmental conditions and differ of course between stages of plant development.

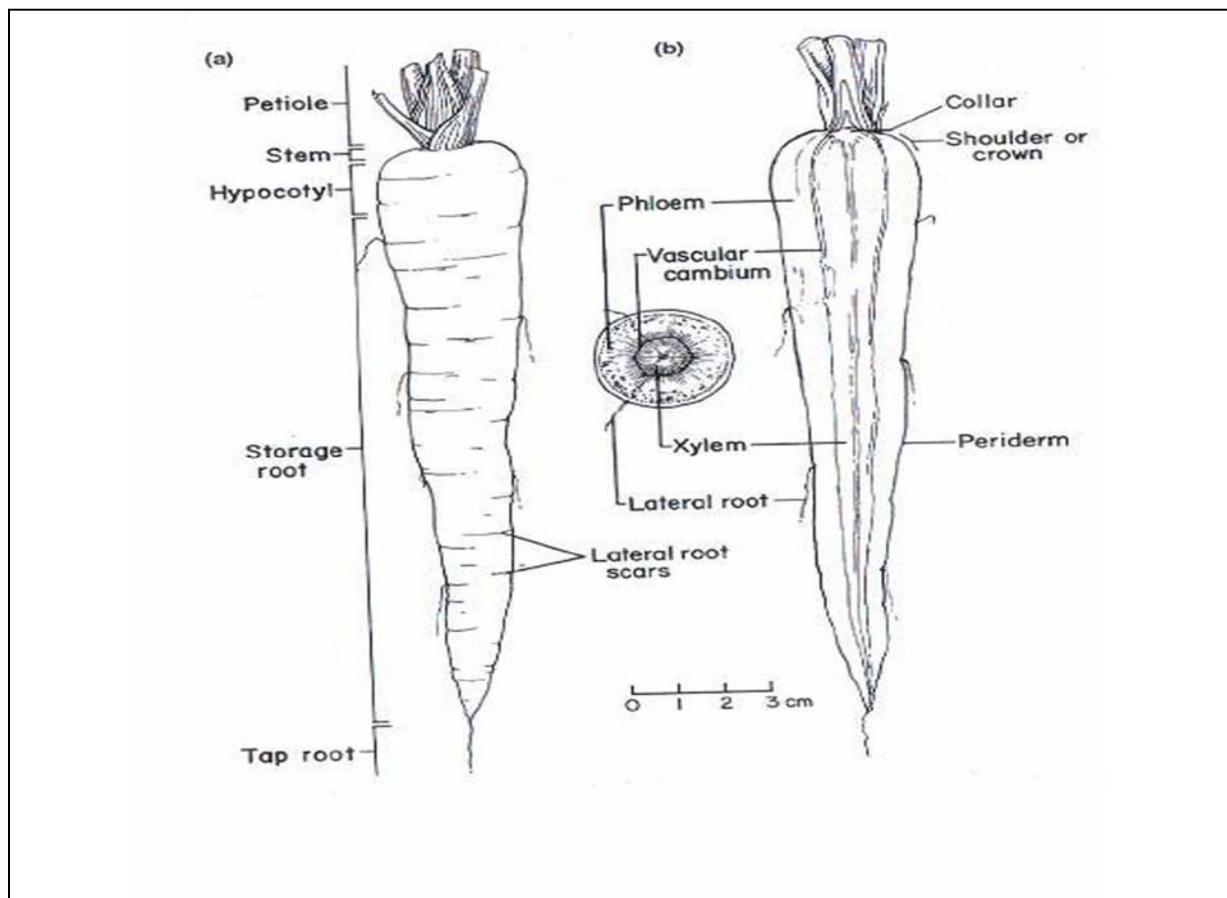


Figure 1. Carrot storage root anatomy¹⁰.

Growth

The taproot develops from the pro-meristem of the embryo. The storage roots of carrots originate from a cylindrical vascular cambium in the hypocotyl and the taproot. At first this cambium consists of separate strips of cells formed from cell divisions between the primary xylem and primary phloem. Thereafter secondary cambium develops between the phloem and the xylem. This cambium extends to form a complete cambial tissue around the central

primary xylem. Here cells are produced that develops to form phloem to the outside and xylem to the inside. These cells expand and differentiate into vessels and storage parenchyma. In carrots, initiation of the secondary cambium usually precedes the development of foliage leaves¹³.

The storage organ is developed largely by secondary growth from the vascular cambium. Due to cell divisions in the xylem and phloem parenchyma considerable carbohydrate accumulation and enlargement occur. During secondary development the taproot apex continues to increase the length of the root. Simultaneously lateral, fibrous, roots develop. These roots do not undergo secondary growth¹⁰.

Oil ducts in the intercellular spaces of the pericycle contain essential oil responsible for the characteristic aroma and flavour of the carrot¹⁴.

The enlargement of the storage root results in shedding of the cortex tissue. The morphological development of the carrot root is shown in figure 2. The surface then becomes covered with periderm originating from the pericycle. Scars marking the exit of the lateral roots appear on this periderm. Wild carrots and primitive cultivars have pronounced root scars on their surface. Fibrous roots are absent on the hypocotyls' portion of the storage root. Fine, highly branched lateral, fibrous roots usually grow from the mid and lower portion of the storage part of the taproot. These roots are usually concentrated within 30 cm of the soil surface¹⁰.

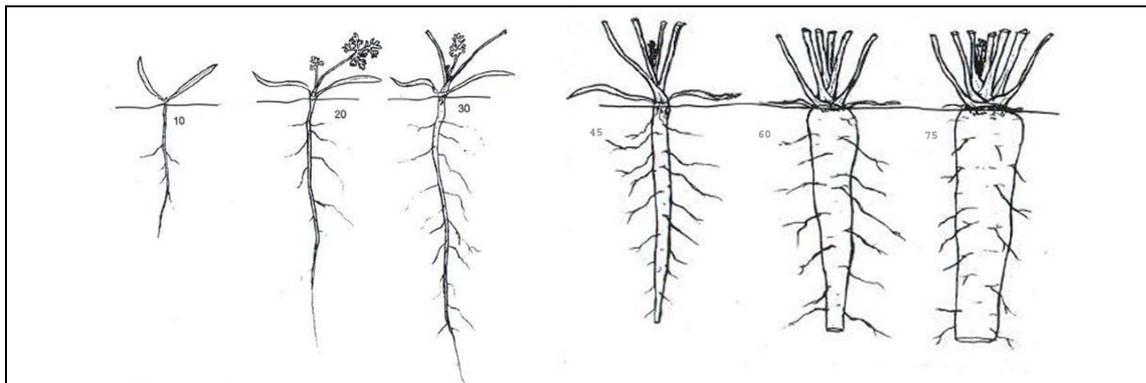


Figure 2. The development of the carrot root 10, 20, 30, 45, 60 and 75 days after planting¹⁰.

The length increase of the storage roots is rapid. Usually it is finalized around 50 days after germination. The growth in length is considerably faster than that of weight. After the first third of the growth period root weight begins to increase. This continues until harvest. The size of the root diameter starts increasing somewhat earlier than the root weight. At the end of the growing season the root weight increases faster than the size of the root diameter measured at the shoulder of the carrot¹⁰.

There are no distinct outer signs of the ripeness of the storage root¹⁵⁻¹⁷. The colour and shape of the root tip are sometimes regarded as a mark for maturity. A pointed tip is more common at the early stages of development. At harvest time the tip is often more blunt. Different shapes of the root tip are shown in figure 3. At early stages the tip is also often paler growing more and more coloured as the harvest approaches.

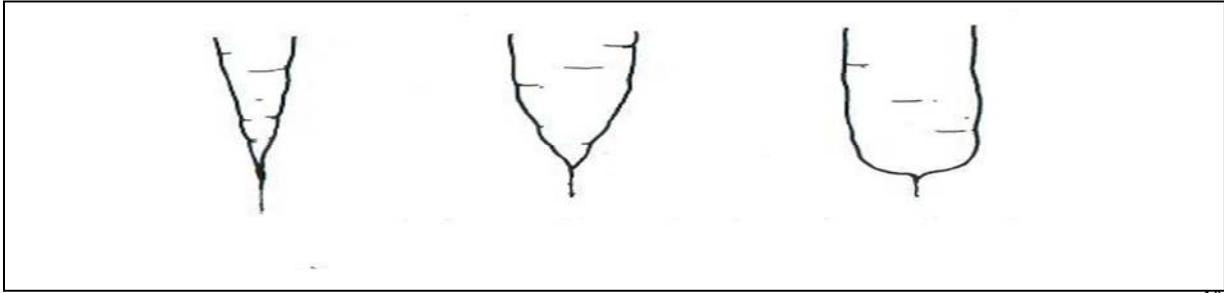


Figure 3. Different shapes of the carrot storage root tip, left pointed tip, right blunt tip¹⁰.

Genetics

Daucus is one of the largest genera in the Apiaceae. It consists of about 25 species. Different species are identified by differences in fruit shape, size, ridges, appendages and ducts. Pollen shape, bract, and leaf characteristics, umbel arrangement and diameter, petal and style size, and chromosome numbers also assist identification¹⁰. Differences in chemical composition, mainly among the phenolics, have been demonstrated useful in distinguish some Daucus species, whereas polyacetylenes, coumarins and sugars have not provided useful distinction^{18, 19}.

The carrot is a diploid plant with nine chromosome pairs. Within the temperate carrot there are several types determined primarily by root shape. An overview of some of these types is given in figure 4.

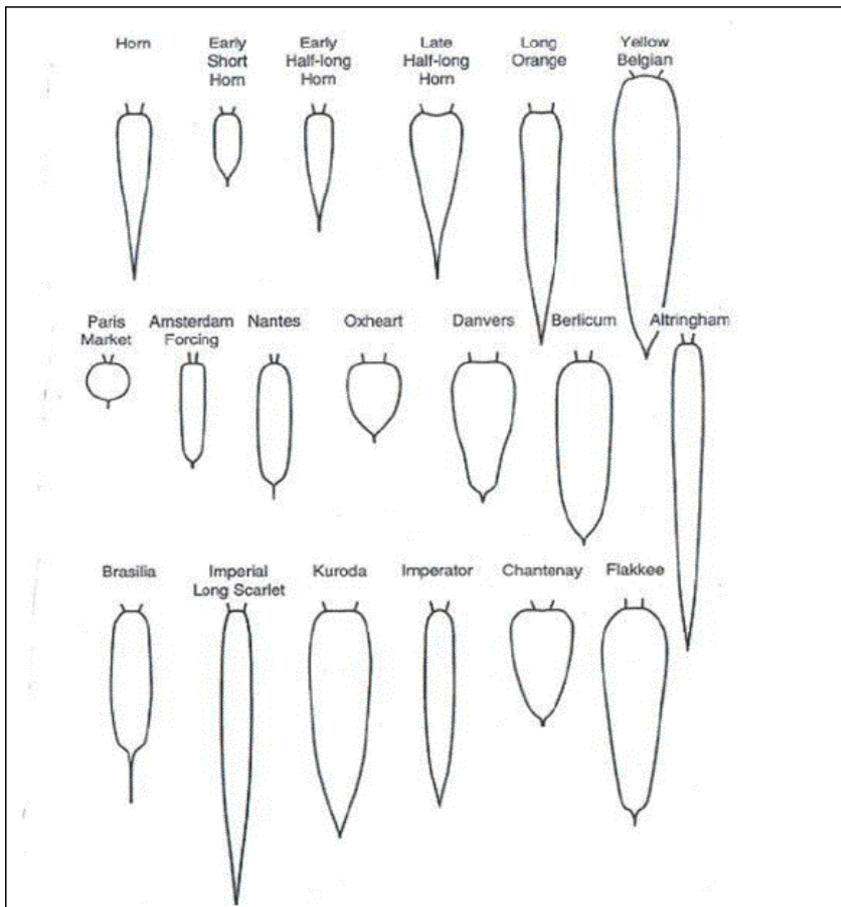


Figure 4. The shape of a collection of carrot varieties¹⁰

Chemical composition

The chemical composition of the carrot storage root varies over time, between cultivars and as a result of cultivation measures. At the time of harvest the carrot storage root consist of about 85 to 90% of water. The rest of the carrot is dry matter. The ash content is usually between 5 and 10% in dry matter²⁰.

The mineral composition of carrot does not show any remarkable features although high content of cadmium can cause problems occasionally, especially on soils low in pH²¹.

Primary metabolites

The amount of different compounds changes during the season. If not noted otherwise the figures mentioned here are collected from the situation at harvest.

Carbohydrates

About half of the dry matter content is soluble sugar. The sugar concentration varies between 30 and 70% of the dry matter. At harvest the sugar content mainly consist of the disaccharide sucrose and the two monosaccharides, glucose and fructose. Measured as percent of the dry matter the sucrose concentration varies between 20 and 45% and the concentrations of the two monosaccharides are about 10% each. Glucose is present both as α - and β -glucose¹⁷.

Maltose has been reported present in carrots but only in quantities lower than 0.5% of dry matter²⁰. Galactose^{20, 22}, lactose and arabinose²⁰ are also found in carrots. Other “sugarlike” compounds reported are glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, nucleosid- mono-, di- and tri- phosphate²³.

Carrots are low in starch. Only seldom does the concentration of starch reach more than 1% in dry matter. The amount of crude fibre is 3-4% on a dry weight basis²⁴.

Nitrogenous compounds

Free nitrogenous compounds accounts for about 1 to 0.5% of the fresh weight. The most important amino acids are aspartic acid, α -alanine, serine, glutamic acid, arginine, valine and threonine. Together with amino sugars, like glucosamine, the free amino acids account for 90% of the total free nitrogenous compounds²³. The amount of protein is between 5 and 10% of the dry matter.

Lipids

The total lipid content in carrot is approximately 0.3% in dry weight. The amount of oil is correlated with the rate of oils ducts in the plant¹⁰. The composition of the carrot oil is complex. It is mainly genetically determined. The amounts of different oils vary however depending on growing condition^{25, 26}.

Organic acids

The total amount of organic acids is about 0.2% in fresh weight²⁷. The most common organic acids in carrots are pyrovatic, oxalic acetic, isocitric and malic acid²⁸.

Secondary metabolites

There is a large amount of secondary metabolites in carrots. The ones mentioned here are more or less connected to the sensory properties, especially taste and flavour.

Vitamins

Carrots are the major single source of provitamin A, as α - and β -carotene, providing more than 17% of the total vitamin A consumption in the US²⁹. Higher levels of carotenoids is normally found in the phloem than in the xylem³⁰.

The carotenoids are commonly divided into two main groups:

1. carotenes or hydrocarotenoids, containing only carbon and hydrogen
2. xanthophylls or oxycarotenoids, the oxygenated derivatives of the carotenes.

Six carotenes has been reported in carrots; α -, β -, γ - and ξ -carotenes, lycopene and β -zeacarotene. The most predominant in orange and yellow carrots are α - and β -carotene³¹. Lycopene is found in red carrots³². Xanthophylls, such as lutein, are common in yellow carrots. In purple carrots we find anthocyanins, belonging to the flavonoids, beside the carotenoids³². An example of the concentrations of carotenoids found in different types of carrots is found in table 3.

Table 3. Concentrations of carotenoids in different types of carrots, nd =not detected³².

Carrot type	Concentrations of carotenoids (mg/100 g carrot, fresh weight)				
	carotenes			xanthophyll	Total
	α -carotene	β - carotene	lycopene	lutein	
Orange	2,2 \pm 0,8	12,8 \pm 3,3	nd	0,26 \pm 0,08	15,2 \pm 4,1
Purple	4,1 \pm 1,2	12,3 \pm 5,1	nd	1,1 \pm 0,73	17,5 \pm 7,0
Red	0,11	3,4 \pm 0,89	6,1 \pm 0,6	0,32 \pm 0,26	9,8 \pm 1,4
Yellow	0,05 \pm	0,18 \pm 0,17	nd	0,51 \pm 0,27	0,71 \pm 0,38
White	nd	0,006 \pm 0,003	nd	0,009 \pm 0,002	0,014 \pm 0,001

The amount of C-vitamin, ascorbic acid, is between 3 and 5 mg/100g fresh weight in orange varieties and about 1 to 2 mg/100g fresh weight in white and yellow varieties³³.

The concentration of vitamin E, in the form of α -tocopherol, is reported to be 0.04- 0.18 ppm, on a dry weight basis. The concentrations are almost the same in the xylem as in the phloem. The concentration of α -tocopherol in the xylem is positively correlated with the concentrations of both α - and β - carotene³⁰.

Volatiles and essential oils

The group of volatile compounds is of great importance for the taste and flavour of carrots. Analyses of carrot roots commonly detect between 30 and 40 volatile substances. Mono- and sesquiterpenes account for about 98% of the total volatile compound mass in carrot³⁴. Other volatile compounds are alcohols, styren, and alkane³³. The number of volatile compounds is determined genetically. The actual amounts of the different volatile compounds is however dependent on the environment³⁵. Two examples of volatile compounds found in carrots are given in figure 5 and table 4.

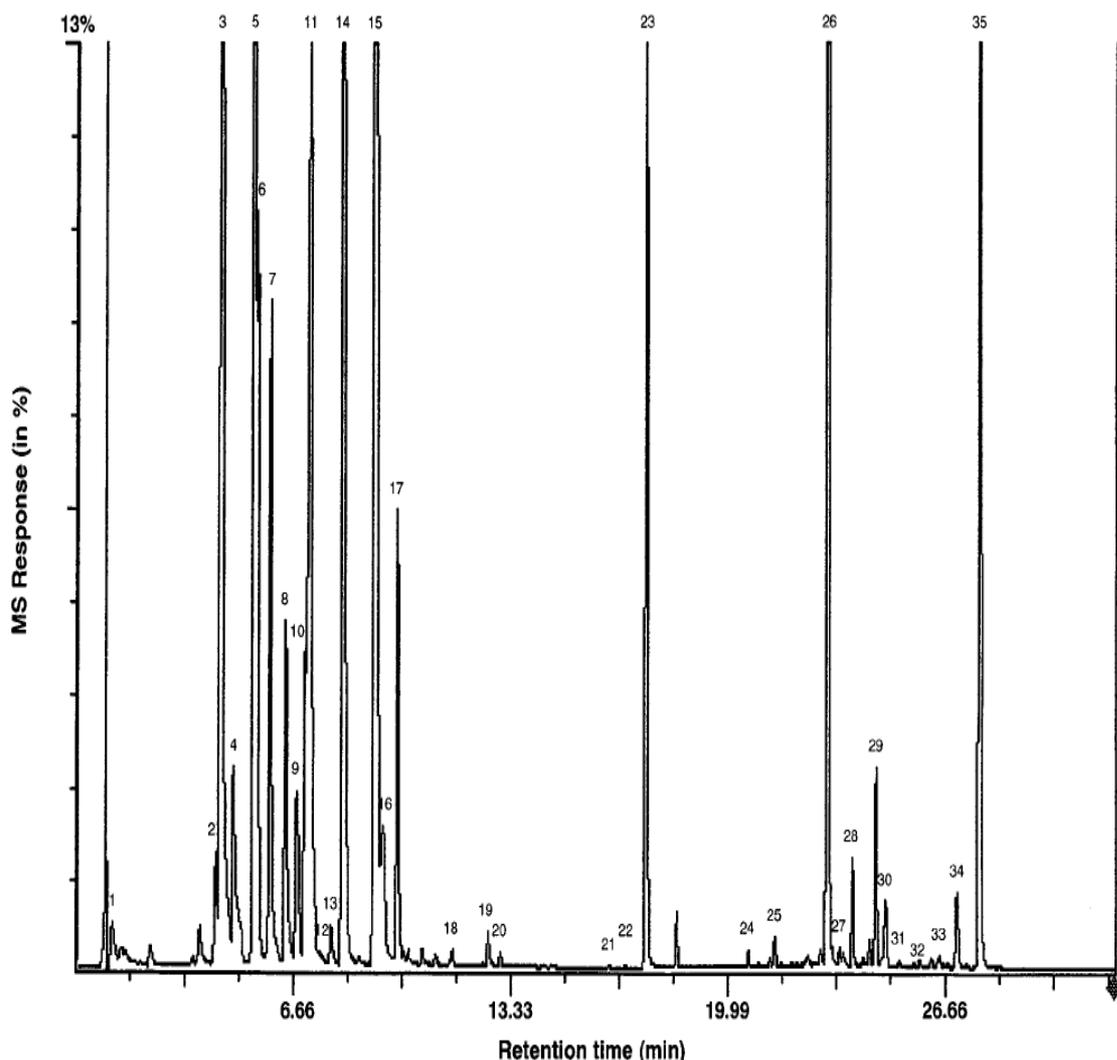


Figure 5. Typical total ion chromatograph of volatile compounds in orange carrots. Peak identification: propanol {1}, α -thujene {2}, α -pinene {3}, camphene {4}, sabinene {5}, β -pinene {6}, myrcene {7}, α -phellandrene {8}, α -terpinene {9}, p -cymene {10}, limonene {11}, cis-ocimene {12}, trans-ocimene {13}, γ -terpinene {14}, terpinolene {15}, 2,5-dimethylstyrene {16}, undecane {17}, camphor {18}, borneol {19}, terpinen-4-ol {20}, linalyl acetate {21}, β -citronellol {22}, bornyl acetate {23}, α -santalene {24}, longifolene {25}, β -caryophyllene {26}, α -selinene {27}, trans- α -bergamotene {28}, α -humulene {29}, cis- β -farnesene {30}, γ -elemene {31}, α -zingiberene {32}, valencene {33}, β -bisabolene {34}, and γ -bisabolene {35}³³.

Table 4. Example of volatiles isolated from four carrot cultivars by dynamic headspace sampling and quantified by capillary GC using LVI technique³⁴.

peak no.	isolated compound ^b	content in ng/50 g/1.5 h ^d					signif. ^e	CV (%) ^f
		RI _{CP-Wax 52CB}	Brasilia	Duke	Fancy	Cortez		
1	α -pinene	1008	4350 c	7780 b	13500 a	4680 c	***	10.3
2	camphene	1044	236 bc	194 c	545 a	270 b	***	9.5
3	β -pinene	1086	799 c	1730 b	2930 a	1630 b	***	11.5
4	sabinene	1105	353 d	2920 c	6220 a	4310 b	***	8.7
5	α -phellandrene	1147	253 a	21 d	220 b	173 c	***	9.3
6	β -myrcene	1153	2960 c	2330 c	13100 a	8750 b	***	12.2
7	α -terpinene	1162	114 c	140 c	188 b	268 a	***	12.5
8	limonene	1183	2430 b	1360 c	3170 a	2120 b	***	10.0
9	β -phellandrene	1191	149 c	193 bc	495 a	250 b	***	10.4
10	γ -terpinene	1230	9050 b	4220 c	12500 a	9070 b	***	11.5
11	(<i>E</i>)- β -ocimene	1241	109 b	68 b	146 b	828 a	***	9.5
12	<i>p</i> -cymene	1252	8190 b	5340 c	17300 a	5280 c	***	11.9
13	terpinolene	1266	26700 a	13500 c	25200 a	17700 b	***	10.7
14	octanal	1274	249 a	280 a	332 a	355 a	ns	16.1
15	6-methyl-5-hepten-2-one	1346	1230 a	1110 a	1660 a	1260 a	ns	17.6
16	unknown (<i>m/z</i> 135, 150, 91, 79, 107, 77, 105)	1376	156 a	142 a	177 a	45 a	ns	43.6
17	unknown (<i>m/z</i> 135, 91, 150, 79, 107, 77, 105)	1389	46 a	51 a	15 b	nd c	***	13.1
18	<i>p</i> -cymene	1411	146 a	105 a	136 a	149 a	ns	25.5
19	unknown monoterpene (<i>m/z</i> 79, 110, 95, 77, 67, 91, 152)	1422	241 a	204 a	231 a	67 b	*	23.7
20	α -copaene	1457	202 a	103 b	29 c	nq c	***	19.9
21	unknown sesquiterpene (<i>m/z</i> 161, 121, 105, 91, 134, 93, 204)	1459	12 a	12 a	18 a	nq a	ns	47.0
22	camphor	1507	nq a	nq a	15 a	nd a	ns	11.5
23	unknown sesquiterpene (<i>m/z</i> 161, 105, 91, 204, 119, 133, 147)	1518	117 c	499 b	1070 a	236 bc	***	26.6
24	bornyl acetate	1574	nq a	nq a	16 a	nd a	ns	23.0
25	β -caryophyllene	1576	20200 b	11500 c	24300 b	40700 a	***	11.3
26	thymol methyl ether	1587	185 a	166 a	nd b	nd b	*	23.8
27	aromadendrene	1622	nd a	nd a	109 a	nd a	ns	21.7
28	(<i>Z</i>)- β -farnesene	1632	nd a	nd a	22 a	nd a	ns	21.7
29	α -humulene	1640	1200 c	740 d	1610 b	2540 a	***	12.4
30	unknown sesquiterpene (<i>m/z</i> 91, 93, 119, 161, 77, 133, 69, 204)	1643	29 b	58 b	128 a	86 a	*	35.0
31	(<i>E</i>)- β -farnesene	1650	117 b	460 a	382 a	465 a	***	14.2
32	valencene	1671	41 d	756 a	315 c	477 b	***	13.1
33	α -terpinyl acetate	1698	36 a	36 a	26 a	nq a	ns	60.0
34	β -bisabolene	1708	440 c	508 c	945 a	731 b	***	10.6
35	(<i>E,E</i>)- α -farnesene	1713	26 a	25 a	47 a	38 a	ns	31.3
36	unknown sesquiterpene (<i>m/z</i> 67, 93, 79, 107, 147, 161, 189, 204)	1722	15 a	13 a	nd b	nd b	***	9.4
37	(<i>E</i>)- γ -bisabolene	1737	5620 c	7160 c	12100 a	10400 ab	**	14.9
38	α -zingiberene ^c	1745	11 c	47 a	32 b	nd c	***	15.6
39	(<i>Z</i>)- γ -bisabolene	1756	276 b	205 b	866 a	949 a	***	16.1
40	β -ionone	1853	51 a	48 a	58 a	25 a	ns	29.4
41	unknown sesquiterpene (<i>m/z</i> 91, 79, 93, 105, 121, 131, 187, 205)	1951	30 a	7.2 b	5.4 b	nq c	***	11.7
42	caryophyllene oxide	1969	303 a	230 a	286 a	350 a	ns	20.8
43	elemicin	2202	6.5 c	6.2 c	23 a	15 b	***	20.7
44	myristicin	2225	12 b	8.4 b	58 a	79 a	***	21.1
	total monoterpenes		56300 b	40300 c	95900 a	55600 b	***	10.4
	total sesquiterpenes		28600 c	22300 c	42200 b	56900 a	***	12.1
	total volatiles		86700 c	64200 d	140400 a	114300 b	***	10.3

^a Sample size injected, 15 μ L. Concentrations of aroma compounds were determined relative to that of the internal standard, (*E*)-2-hexen-1-ol. ^b MS and GC retention indices (RI) were consistent with those of reference compounds unless noted. MS of unknown compounds are listed in parentheses with descending intensities of fragment ions. ^c Tentatively identified. No standard available but the MS was consistent with published data (32, 33). ^d nq, not quantified (less than 5 ng/50 g/1.5 h) and nd, not detected. A minimal content of 0 ng/50 g/1.5 h was assigned to facilitate statistical analysis. ^e Significance: ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. ^f Mean coefficient of variance (CV) for three replicates of each cultivar.

The most frequent essential oils are the monoterpenes; sabinene, β -myrcene, α -terpinolene and β -caryophyllene together with some sesquiterpenes³⁶.

Terpenes

The terpenes are aromatic compounds occurring naturally in carrot mostly as mono- and sesquiterpenes. Usually between 17 and 20 different simple terpenes contribute to the typical carrot flavour³⁷⁻³⁹.

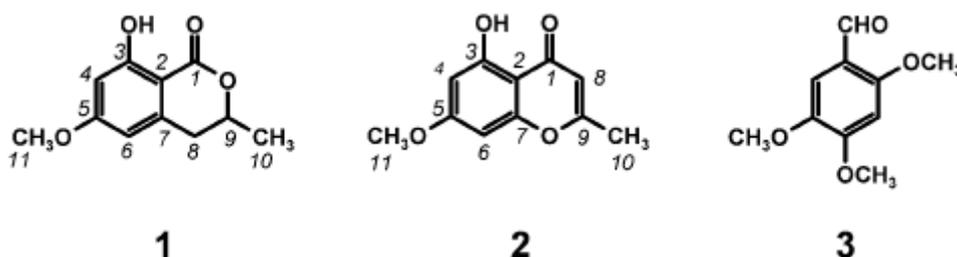
Phenols

Phenols are biosynthesised along the polyketide (acetylcoenzyme A) or the shikimic pathway. The most common phenolic substances in carrot are hydroxycinnamic acid derivatives. Also caffeic acid, isochlorogenic acid and chlorogenic acid are found in carrots^{33,40}. The amount of the most common phenols found in carrots of different colour is listed in table 5.

Table 5. Some phenolic compounds in different carrot varieties³³.

compound	levels of phenolic compounds (mg/100 g)			
	orange	purple	yellow	white
3'-caffeoylquinic acid	0.28 ± 0.02 ^d	0.88 ± 0.05 ^c	0.09 ± 0.01 ^e	0.09 ± 0.01 ^e
<i>cis</i> -3'-caffeoylquinic acid	nd ^g	1.94 ± 0.10	nd	nd
5'-caffeoylquinic acid	8.50 ± 0.24 ^d	54.08 ± 3.10 ^c	4.41 ± 0.21 ^e	4.47 ± 0.20 ^e
caffeic acid	nd	2.42 ± 0.16	nd	nd
3'- <i>p</i> -coumaroylquinic acid	0.54 ± 0.02 ^d	0.91 ± 0.06 ^c	0.20 ± 0.02 ^f	0.31 ± 0.02 ^e
3'-feruloylquinic acid	0.21 ± 0.02 ^{def}	7.30 ± 0.20 ^c	0.19 ± 0.01 ^f	0.26 ± 0.02 ^{de}
3',4'-dicaffeoylquinic acid	2.08 ± 0.15 ^d	2.78 ± 0.18 ^c	1.30 ± 0.07 ^e	1.06 ± 0.06 ^f
5'-feruloylquinic acid	0.11 ± 0.01 ^f	0.96 ± 0.03 ^c	0.51 ± 0.03 ^d	0.39 ± 0.02 ^e
<i>cis</i> -5'-caffeoylquinic acid	nd	0.49 ± 0.02	nd	nd
5'- <i>p</i> -coumaroylquinic acid	0.13 ± 0.01 ^d	0.74 ± 0.03 ^c	0.11 ± 0.01 ^d	nd
4'-feruloylquinic acid	0.40 ± 0.03	nd	nd	nd
3',5'-dicaffeoylquinic acid	3.80 ± 0.20 ^c	0.44 ± 0.02 ^f	0.75 ± 0.02 ^e	1.74 ± 0.09 ^d
3',4'-diferuloylquinic acid	0.07 ± 0.01 ^e	0.53 ± 0.03 ^c	0.12 ± 0.01 ^e	0.31 ± 0.02 ^d
3',5'-diferuloylquinic acid	0.09 ± 0.01 ^d	1.17 ± 0.02 ^c	0.04 ± 0.01 ^d	0.06 ± 0.01 ^d
total phenolics	16.21 ± 0.21 ^d	74.64 ± 3.32 ^c	7.72 ± 0.22 ^e	8.69 ± 0.24 ^e

When discussing bitter taste some specific phenolic substances are often mentioned. The structure of 6-methoxy-mellein and two other of these substances found in carrots are given in figure 6.



- 1) 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (6-methoxymellein)
- 2) 5-hydroxy-7-methoxy-2-methylchromone (eugenin),
- 3) 2,4,5-trimethoxybenzaldehyde (gazarin).

Figure 6. Structures of phenolic compounds, described as bitter-tasting in carrots⁴¹.

The phytoalexin, 6-methoxymellein, 6MM (3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin), is a secondary metabolite that inhibits the growth of many microorganisms. It is elicited in carrot root tissues inoculated with fungi, as well by treatment with various elicitors⁴²⁻⁴⁶. It is also induced by numerous mold species^{45, 47} by exposure to UV light⁴⁸ (Mercier, Arul et.al., 1994), and from pectinolytic enzymes^{49, 50}. However, exposure to ethylene appears to be the most common stimulus for its formation in carrots⁵¹.

Polyacetylenes

Food plants of the Apiaceae plant family such as carrots, celery and parsley, contain a group of bioactive aliphatic C₁₇-polyacetylenes. They form a distinct group of relatively chemically

reactive natural products. More than 1400 different polyacetylenes and related compounds have been isolated from higher plants⁵². Aliphatic C₁₇-polyacetylenes of the falcarinoltype are common in the families Apiaceae and Araliaceae^{53, 54}. Polyacetylenes of the falcarinol-type are formed from oleic acid by dehydrogenation leading to the C₁₈-acetylenes crepenynic acid and dehydrocrepenynic acid, which is then transformed to C₁₇-acetylenes by β-oxidation. Further oxidation and dehydrogenation leads to falcarinol and related C₁₇-acetylenes of the falcarinol-type^{53, 54}.

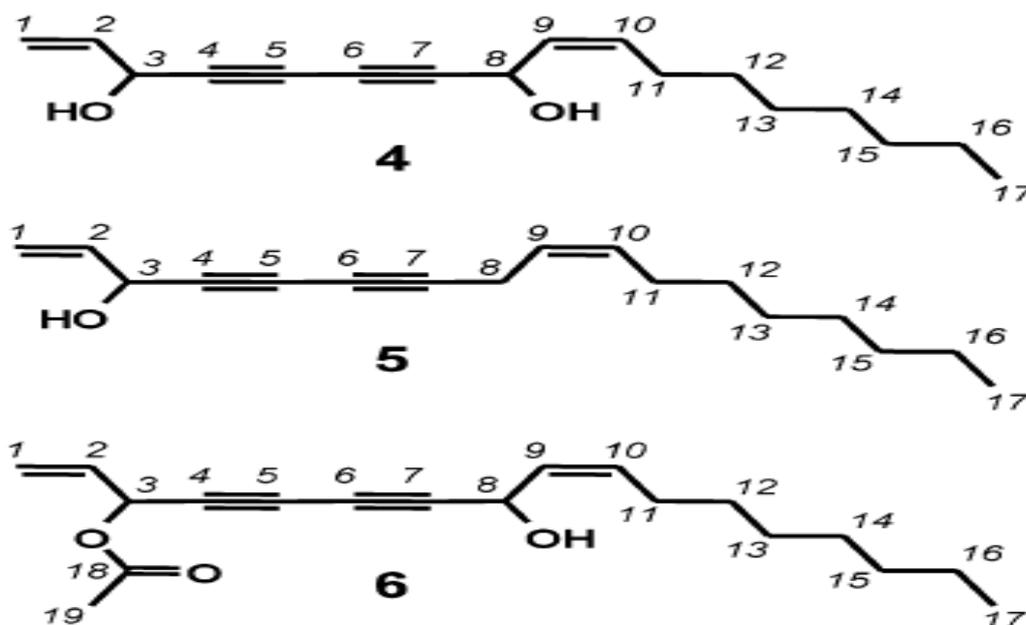
Three polyacetylenes from the falcarin-group are often mentioned in connection with carrots:

- (Z)-heptadeca-1,9-diene-4,6-diin-3,8-diol (falcarindiol, FaDOH),
- (Z)-heptadeca-1,9-diene-4,6-diin-3-ol (falcarinol, FaOH),
- (Z)-3-acetoxyheptadeca-1,9-diene-4,6-diin-8-ol (falcarindiol 3-acetate, FaDOAc).

Falcarindiol, FaDOH, has recently been detected as a bitter tasting constituent in carrot⁵⁵. There are some results showing that FaDOH is part of the defence against fungal infection⁵⁶.

Falcarinol, FaOH, is a bioactive metabolite. It has shown cytotoxic activity against human tumour cells in vitro⁵⁷ and possibly also in vivo⁵⁸. FaOH stimulates differentiation of mammalian cells down to 1 ng/ml and shows toxic effects above 1000 ng/ml⁵⁹. Furthermore FaOH is said to have anti-inflammatory⁶⁰ and anti-tuberculosis⁶¹ effect. It also causes allergic dermatitis after skin exposure⁵⁴. When eating carrot juice containing falcarinol the concentration in human blood plasma increases within half an hour after ingestion, reaches its maximum approximately 4 hours after and goes back to starting level after 8 hours⁶².

Falcarindiol 3-acetate, FaDOAc, has not yet been connected with any specific task. The chemical structure of three of the polyacetylenes from the falcarin-group is shown in figure 7 on the next page.



4) (Z)-heptadeca-1,9-diene-4,6-diin-3,8-diol (falcarindiol, FaDOH)
 5) (Z)-heptadeca-1,9-diene-4,6-diin-3-ol (falcarinol, FaOH)
 6) (Z)-3-acetoxyheptadeca-1,9-diene-4,6-diin-8-ol (falcarindiol 3-acetate, FaDOAc).
 Figure 7. Structures of different polyacetylenes⁴¹.

Taste perception

The following description of the human taste perception is a brief presentation collected from the internet⁶³.

The perceptions in connection with a meal involve many senses. Sight, touch, warmth, smell and taste interact to give us an impression of the food we are to eat. The experience of a meal is also influenced by psychological factors such as dining room atmosphere and other guests at the table.

The starting point of the taste analysis is the solution produced when by chewing we mix food and saliva with each other. Special glands in the mouth pit produce the saliva. It is secreted under the tongue, beside the second molar in the upper cheek, and from the walls of the mouth pit. There are two types of saliva. The mucous saliva makes the food easier to swallow. The serous saliva dilutes the food and also contains enzymes that hydrolysis starch into sugar. The perception of sweet taste is often dependent on the activity of such enzymes.

The taste organ

In mammals taste buds are located throughout the oral cavity, in the pharynx, the laryngeal epiglottis and at the entrance of the oesophagus. Taste buds on the dorsal lingual epithelium are the most numerous. On the tongue taste buds are contained within four major classes of papillae. The anatomy of the human tongue is shown in figure 8.

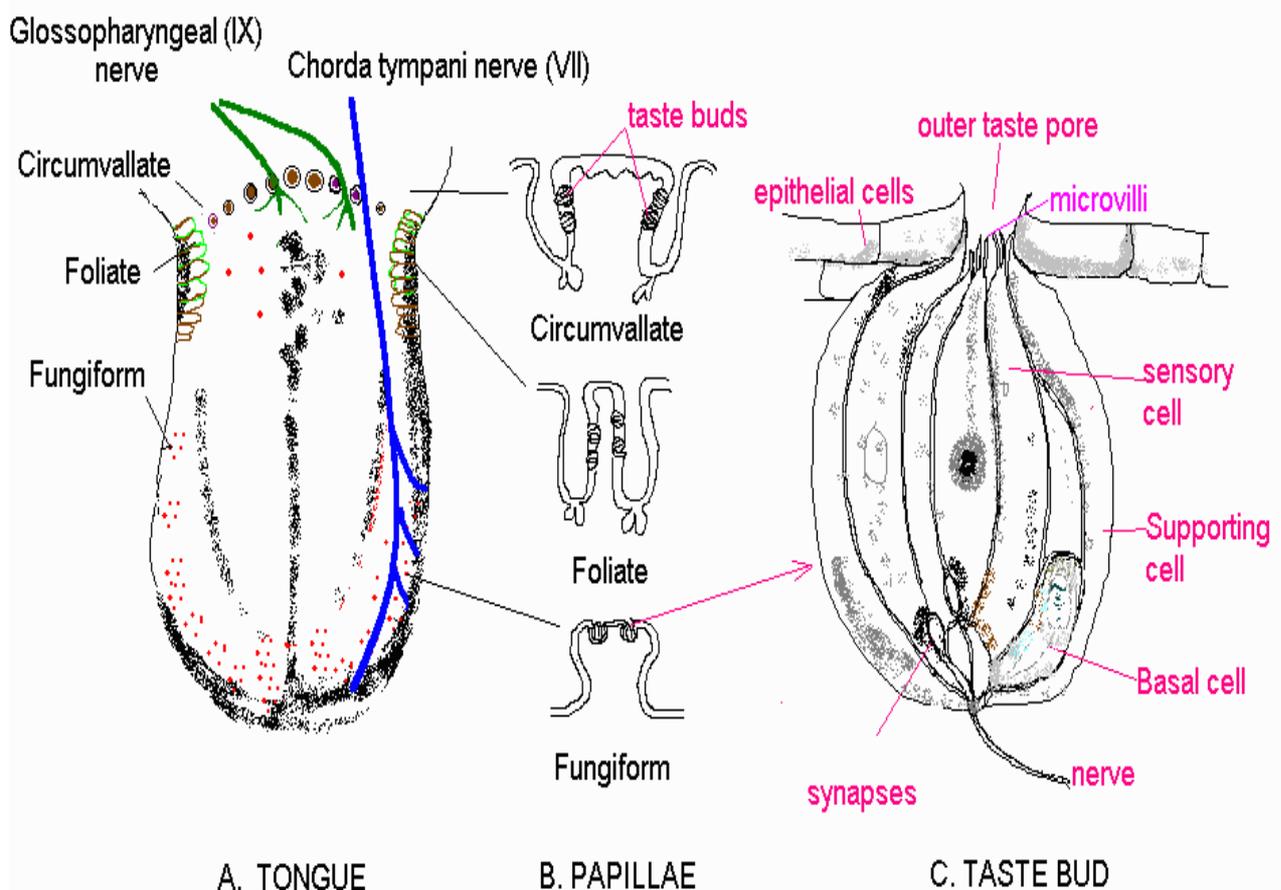


Figure 8. The human tongue as the organ of taste⁶³

- *Fungiform papillae* are located on the most anterior part of the tongue and generally contain one to several taste buds per papilla. They are innervated by the chorda

tympani branch of the facial (VIIth cranial) nerve. They appear as red spots on the tongue - red because they are richly supplied with blood vessels. The total number of fungiform papillae per human tongue is around 200. Papillae at the front of the tongue have more taste buds (1-18) compared to the mid-region (1-9). It has been calculated that there are 1120 fungiform taste buds per tongue.

- *Foliate papillae* are situated on the edge of the tongue slightly anterior of the circumvallate line. They are predominantly sensitive to sour tastes. Innervated by the glossopharyngeal (IXth cranial) nerve. On average 5.4 foliate papillae per side of the tongue, 117 taste buds per foliate papillae, total = 1280 foliate taste buds per tongue.
- *Circumvallate papillae* are sunken papillae, with a trough separating them from surrounding wall. The taste buds are in tiers within the trough of the papillae. They are situated on the circumvallate line and confer a sour/bitter sensitivity to the posterior 2/3 of the tongue. Innervated by the glossopharyngeal (IXth cranial) nerve. 3-13 circumvallate papillae per tongue with 252 taste buds per papillae, total = 2200 circumvallate taste buds per tongue
- *Filiform papillae* are mechanical and non-gustatory.

In addition there are 2500 taste buds on the epiglottis, soft palate, laryngeal and oral pharynx. The number of taste buds declines with age.

Different taste qualities

There are five basic tastes: salt, sour, sweet, bitter and umami.

1. *Salt taste*

Na^+ ions enter the receptor cells via Na-channels. These are amiloride-sensitive Na^+ channel. The entry of Na^+ causes a depolarization, Ca^{2+} enters through voltage-sensitive Ca^{2+} channels, transmitter release occurs and results in increased firing in the primary afferent nerve.

2. *Sour taste*

H^+ ions block K^+ channels. K^+ channels are responsible for maintaining the cell membrane potential at a hyperpolarized level (close to the K^+ equilibrium potential of around -85mV). Block of these channels causes a depolarization, Ca^{2+} entry, transmitter release and increased firing in the primary afferent nerve.

3. *Sweet taste*

There are receptors in the apical membrane that bind glucose and other saccharides. Binding to the receptor activates adenylyl cyclase, thereby elevating cAMP. This causes a PKA-mediated phosphorylation of K^+ channels, inhibiting them. Depolarization occurs, Ca^{2+} enters the cell through depolarization-activated Ca^{2+} channels, transmitter is released increasing firing in the primary afferent nerve.

4. *Bitter taste*

Bitter substances cause the second messenger (IP_3) mediated release of Ca^{2+} from internal stores (external Ca^{2+} is not required). The elevated Ca^{2+} causes transmitter release and this increases the firing of the primary afferent nerve.

5. *Umami taste*

Umami is the taste of certain amino acids (e.g. glutamate, aspartate and related compounds). Recently it has been shown that the *metabotropic* glutamate receptor (mGluR4) mediates umami taste. Binding to the receptor activates a G-protein and this may elevate intracellular Ca^{2+} .

The chemical processes connected to the perception of these primary taste qualities are briefly presented in figure 9 on the next page.

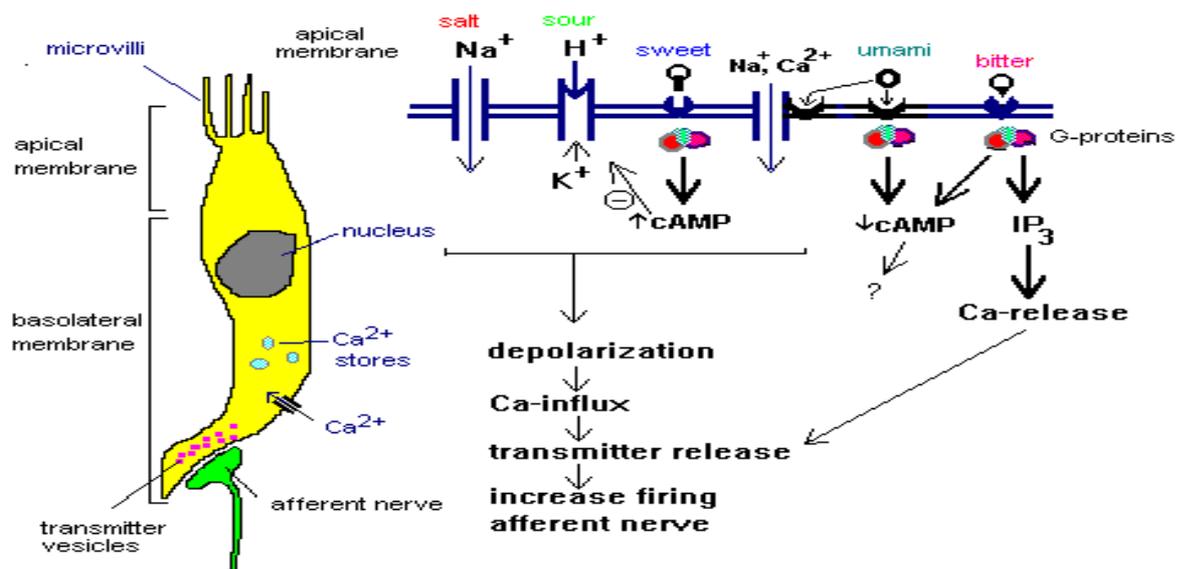


Figure 9. Schematic description of a taste receptor cell and the chemical processes connected to the five taste qualities⁶³.

Sensory evaluation

Methodology

There are different types of sensory evaluation methods⁶⁴. An *affective evaluation* describes the assessors liking or disliking of a product. The discriminant evaluations focus only on if there are differences between products. The *analytical, or descriptive, evaluation* tries to describe the properties of the product or the differences between products.

Often the context of the testing situation interplays with the assessors' evaluation. The temperature, colour and sound level of the testing room are important factors, as is the protocol that is been used.

There are different standards describing possible ways to do a sensory evaluation. ISO 5492 describes terms to use, ISO 6564 lists different properties of an aroma profile. ISO 8589 describes the testing room, ISO 3972 the training of the panellists and so on. Besides the ISO system there are other standards describing the same issues. ASTM E-2454 and ASTM E - 1885 both describe different types of sensory testing methods. More information on different standards can be found on www.ansi.org, www.iso.org and www.astm.org.

The first step is to “calibrate” the senses. This can be done in at least two ways. One way is by tasting standardised solutions^{41, 65}. This makes it easier to compare the result from one test to another. But at the same time it makes the resolution smaller because the terms used has nothing to do with for example carrots. This method is therefore applicable in test where a limited number of properties, like sweetness and bitterness, are to be evaluated. An other way is to screen the products to be evaluated and after that decide the appropriate terms to use⁶⁶⁻⁷⁵. Concerning carrot this means that the panellists start by tasting all the different carrot samples included in the test. They then discuss their experiences and agree on a common set of terms to use during the “real” test. Each term is evaluated separately, often on a linear scale. The advantage of this procedure is a better resolution in the description and a better discrimination

between the samples. The total taste profile is also more detailed using this method. The disadvantage is difficulties to compare one test with another.

The most frequently used discriminant test is the triangle test. Three samples, of those are two the same, are presented to the assessor. The assessor has to recognise the single sample and describe the difference. In a pure descriptive test the properties of the carrots are described more or less independent from each other.

Assessors

The evaluating person can be a trained panellist or an untrained consumer. In both cases the personal set of values of the assessors can influence the result of the evaluation.

In *untrained panellist test* the assessors are mostly picked by random. Most consumers test uses this kind of panels together with an affective method and hedonic ratings. The hedonic rating concerns “the liking, the immediate, qualitative, affective evaluation of a food; the degree of experience of pleasure or displeasure”⁷⁶. The assessor has often to relate his opinion to a linear scale. The most commonly used scale is the 9-point scale⁷⁷. The results from consumers test often vary a lot. Most tests involve more than 100 assessors to enable an appropriate statistical evaluation.

The number of assessors in a *trained panel test* is often under 10. The panel mostly uses an analytical method in order to describe the product. The terms to use in this description are often standardised and the first step in a panel test is often to screen the material as mentioned earlier.

One important problem is the way the panellist uses the scale. Some of them use the whole scale and the numbers between different samples vary a lot. Other panellist uses only a part of the scale. In one way or the other the difference in variation between the panellists must be dealt with when making a statistical evaluation.

Terms used in sensory evaluation of carrots

There are standardised sets of terms to use when making sensory analysis, for example ISO 5492. However many other terms have been used during the years. To get an overview some of the terms are listed here, arranged “sense by sense”.

The sense of sight gives us experiences of colour. Together with other senses this also makes it possible for us to perceive shape (form). Terms used in connection with sight are: *whiteness, colour, colour hue, colour strength, discoloration, freshness, shape, cylindricity, appearance and bluntness*. Sometimes these properties are called outer quality.

Our nose can perceive the flavour of a carrot before we put the carrot into the mouth. The impression of flavour also comes to the nose via the mouth pit. Terms used in connection with flavour are: *overall, intensity, carrot, sharp, green, turpentine, diesel, petrol, ethanol, cardboard, earthy, fruity, fresh, musty, stale, nutty, sweet, bitter, burning, pungent, harsh, and flowery*.

The tactile properties of a carrot can be recognised by our hands, lips, teeth, tongue or palate. There are different terms used to describe this side of our food experience; *firmness, crispiness, juiciness, soapiness, oiliness, woodiness, crunchiness, texture, chewy, finger feel, mouth feel, hardness, moistness, spongy, toughpacking and toughness*.

The taste qualities are perceived in the oral pit as an experience of the liquid created in the mouth when chewing. Terms used when describing this experience are; *sweet, sickingly sweet, sour, acidic, bitter, salty, intensity, aftertaste, green, foliage, terpene, earthy, peppery, carrot, overall, preference, fruity, turpentine and harsh*,

Some of the terms mentioned also involve our sense of warmth, or temperature. Examples of such terms are; *peppery, burning, pungent, sharp, turpentine and harsh*.

Sweet and bitter taste in carrots

Sweetness is one of the most appreciated features of carrot taste⁷⁸. Although sometimes the sweetness can be almost sickening⁷³, most consumers liking is positively correlated with the perceived sweetness of carrots^{66, 67, 75, 78, 79}.

Bitter taste has been a positive topic in medicine for a long time⁸⁰. In carrots it is more of a problem. Supertasters are very sensible to bitter taste⁸¹. Also children are more sensitive and often dislike bitter tasting food⁸². One reason for these differences is probably the fact that children and supertasters have more tastebuds⁸³. As both of these groups have a considerable impact on the preferences on our food⁸², they might have contributed to the attempts of finding more sweet tasting carrots.

Bitter taste is one of the main reasons for low quality score of carrots⁸⁴. The term “bitter” has been supplemented with the term “harsh”. This term is used to describe a burning turpentine-like flavour occurring most clearly at the back of the throat⁷⁸. It is sometimes hard to draw a line between these two terms. They are however both well documented in carrot research^{33, 66, 68, 70, 73-75, 78, 85-94}.

There are differences between carrot varieties both in sweetness and bitterness. This has made it possible for the carrot breeder to influence the bitter and sweet taste of carrots. During the last decades carrots has lost some of their harsh features and gained an increasing sweetness⁸⁸.

Sweet and bitter tasting compounds

The sweetness in carrots is commonly related to sugars. Fructose is considered to have the highest relative sweetness of the three major sugars in carrot⁹⁵. If the sweetness of sucrose is set to 1 the relative sweetness of fructose is 1.75 and of glucose is 0.75⁹⁶. Some of the terpenes, myrcene³⁶ and perhaps also terpinolene,⁷³ is described as slightly sweet tasting. The influence from the terpenes or other substances besides sugars, on the total sweetness of the carrot must be regarded as low.

Terpenes are also regarded as an important group concerning the harsh and bitter tastes in carrot,^{39, 97, 98}. Harsh, turpentine-like flavours are reported associated with the presence of the volatiles, particularly γ -terpinene and total volatiles, and a reduction in sugars. The reverse is found for sweetness and overall preference that both are enhanced by sugars and diminished by volatiles. Overall carrot flavour is heightened by a reduction in total volatiles. Sucrose levels correlate positively and reducing sugars negatively with volatile terpene levels⁹⁷. Volatile terpenoid levels above 35-40 $\mu\text{l/l}$ seems to be associated with the characteristic harsh flavour whereas terpenoid content below 10 $\mu\text{l/l}$ diminishes the characteristic carrot flavour and causes a flat taste⁹⁹.

As already been indicated there are interaction between terpenoids and sugar^{74, 87, 99}. Sweeter taste in carrots grown in northern parts of Scandinavia^{100, 101} is stronger correlated to a lower

concentration of terpenoids than to increased levels of sugar, ⁷⁴. The more harsh taste of stored carrots does not correspond to an increase of terpenoids during storage but to a decrease in the sugar content ^{73, 87, 99}. The fact that frozen carrots tastes sweeter can partly be a result also of losses of terpenoids in the freezing and thawing process ⁷⁴.

High scores on the properties oiliness, cut carrot foliage and petrol is related to high concentration of terpenes in the carrot while the attributes bitterness, soapiness, woodiness and fruitiness properties are assumed not to be connected with the concentration of terpenes ³³. High positive correlation between terpenes (α -terpinene, β -myrcene, trans-caryophyllene, farnesene, α -humulene) and the sensory variables terpene flavour, green flavour, earthy flavour, bitter taste and aftertaste is found in an another study where it was concluded that these terpenes are responsible also for bitter taste and thus suppressed the perception of sweet taste in carrots, ³⁵. Terpinolene probably plays only a minor role in masking sweet taste in carrots, ³⁵.

A wide range of different compounds has been mentioned in connection with bitter taste in carrots. L-tryptofan is considered to be the amino acid tasting most bitter ¹⁰². A bitter tasting glycoside has been detected in carrot leaves ¹⁰³ but not in the roots. The complete identification of this compound is however not clear ¹⁰⁴.

The compound gazarin, or 2,4,5-trimethoxybenzaldehyde, has been isolated from carrot seeds ¹⁰⁵. It is reported as bitter but no connection to the taste of the carrot root has been documented ⁷³. It has been found in very small amounts in carrot roots and its bitter taste threshold concentration has been estimated to 36 ml/l water ⁴¹.

Chlorogenic acid is the most common phenolic in carrots. Together with some other hydroxycinnamates it is described as having a mild bitter taste ¹⁰⁶. The concentrations of these compounds are normally too low to allow their bitterness to be detected ¹⁰⁷.

More than 50 years ago it was detected that hydrocarbon extracts from bitter tasting carrots had a different UV-absorption spectra ⁸⁵. Since then one trail of the search for bitter principles in carrots has been pointing towards the two phenolics; 6-methoxymellein and eugenin.

Many reports have been published on 6-methoxymellein, 6MM ^{42, 46, 47, 108-113}. Sensory analysis have not been able to find a clear correlation between bitterness and the concentration of 6MM in carrots ^{44, 73, 114}. Studies on purified 6MM has revealed a bitter taste threshold concentration of 100 mg/kg carrot ⁹¹. The bitter taste threshold concentration in water has been estimated to 20 ml/l water. This is higher than the concentrations found in stress induced bitter tasting carrots ⁴¹.

Also in connection with eugenin the results are contradicting ^{38, 41, 43, 72, 115}. Bitter taste has been shown correlated with the amount of eugenin ^{44, 114}, while others report no such connection ⁴⁰. The bitter taste threshold concentration in water for eugenin has been calculated to 72 mg / l water ⁴¹.

Some reports have attributed the presence of bitter taste to the concentration of water soluble phenolics ^{72, 115}. No such particular substance has yet been identified ⁴¹.

Another trail has put the focus on the polyacetylenes ^{52, 54, 56, 112, 116-120}. The bitter taste detection threshold for the falcarinols found in carrots has been estimated to 10 mg FaDOH/ l water, 20 mg FaOH/ l water and 60 mg FaDOAc/ l water. The latter of these substances

exhibited a burning, harsh sensation already at 15 mg/ l water ⁵⁵. A correlation between the concentration of FaDOH and the bitter taste of carrots, especially in carrot puree, has been reported ⁵⁵.

The development of sweet and bitter taste

Sweet and bitter taste in carrots appears to develop differently in time and on different location.

Different parts and tissues

Sweet taste is often more pronounced in the centre and lower (tip) part of the carrot. The phloem is mostly sweeter than the xylem ⁷⁴. Bitter taste is more often detected in the upper (crown) part and more strongly connected to the phloem than the xylem ^{55, 74}. The sugars are mainly stored in vacuoles in the parenchymatic tissues ^{16, 17}. The total sugar content does not differ much between different parts of the carrot ⁷⁴. The amount of sucrose is higher in the upper part and in the phloem. Monosaccharides, especially fructose are more common in the centre and lower, tip, part of the carrot and in the xylem ^{36, 74}.

Terpenes are more common in the upper part of the carrot and are evenly spread between phloem and xylem. Terpinolene is an exemption being more evenly spread also in the lower part of the carrot and showing higher concentrations in the phloem ⁷⁴. 6MM are more concentrated to the phloem all along the carrot ⁵⁵. Polyacetylenes are more common in the upper part of the carrot and in the phloem, although falcarinol and falcarindiol-3-acetate is more evenly distributed also in the xylem, the later even more concentrated in the xylem, ⁵⁵. The levels of polyacetylenes reported as a mean of 16 carrot cultivars grown in Sweden is presented in table 8.

Table 6. Levels of polyacetylenes in carrot cultivars grown in Sweden⁵⁶.

Substance µg/g fresh weight	In the peel		In the phloem	
	Mean	Variation	Mean	Variation
Falcarindiol	57	39-92	11	6-19
Falcarinol	3	1-5	6	3-12

During the season

Regarding sugar the development of a carrot crop is divided into three phases: During the first no soluble sugar is stored, in the second phase only reducing sugars are stored and in the third phase mainly sucrose is stored in the taproot ¹²¹. The shift into phase three probably takes place about 50 days after sowing ¹²². This coincides with an increased sink strength of the carrot storage root ^{123, 124}. The decline of the reducing sugar content may be caused by a continuous reduction of acid invertase activity ¹²⁵. The accumulation of sucrose in the taproot seems to be more influenced by environmental factors than the storage of reducing sugar ⁷⁴.

The amount of 6MM, falcarindiol and falcarindiol-3-acetate decreases with increasing root weight while the amount of falcarinol does not seem to be affected by root weight ¹¹⁸.

Factors influencing sweet and bitter taste in carrots

The following factors are supposed to influence the taste of carrot and are the ones most commonly found in literature.

Variety

The genetic factor is one of the most important sole factors related to the taste properties of carrot⁸⁷. The flavour profile from carrots of different colour reveals big differences, is illustrated in figure 10.

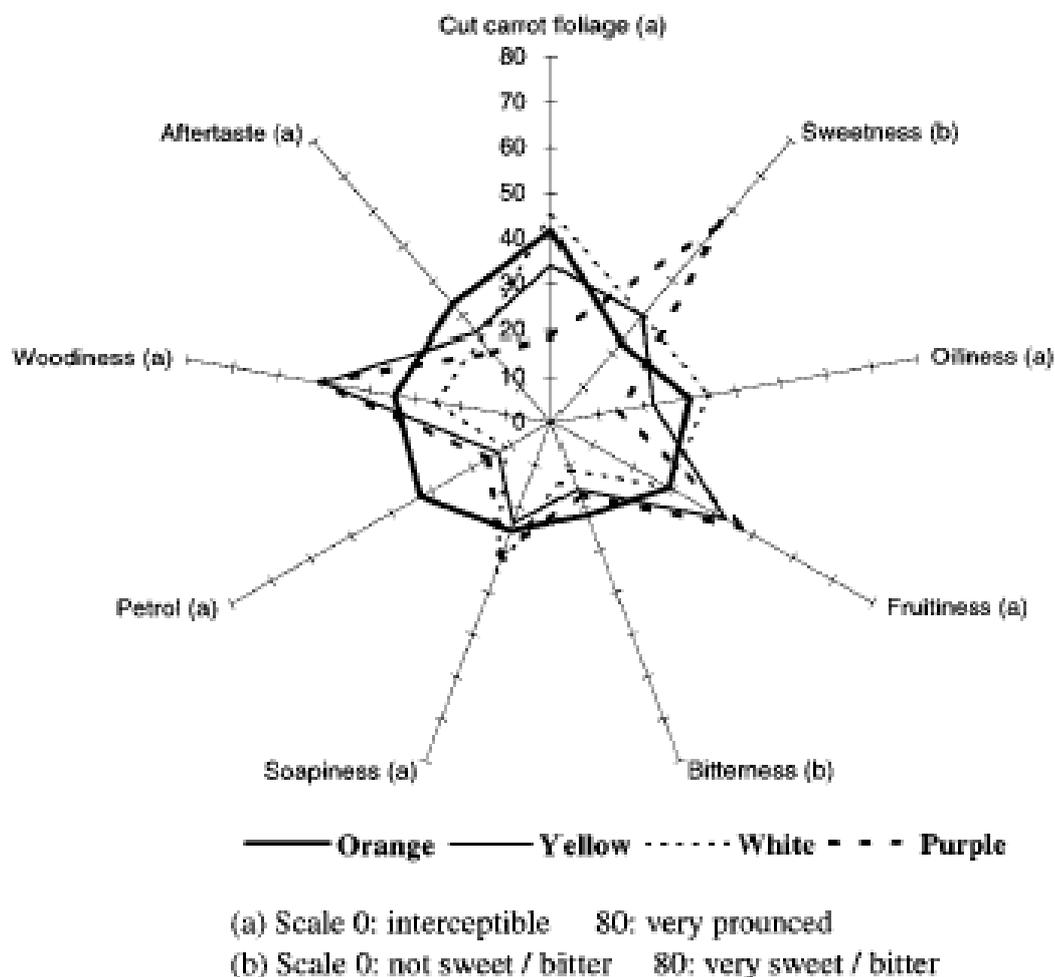


Figure 10. Flavour profile analysis of coloured carrots varieties³³.

Purple carrots score high on sweetness, fruitness, soapiness and woodiness and low in oiliness and cut carrot foliage flavour. White carrots are more or less opposite to the purple varieties and yellow and orange carrots score somewhere in between. Orange carrots score high on petrol, after taste and bitterness³³.

However when comparing varieties of the same type the differences sometimes are not so big, A Danish study reports the properties of the variety “Bolero” to differ significantly from the other five varieties, also from the Nantes type. The properties among the five other Nantes varieties did not differ so much from each other⁷⁵.

Significant differences are reported between varieties concerning sweetness and bitterness^{75, 92, 93}.

The composition of volatiles is also dependent on variety^{36, 37, 88, 126}. Out of 36 volatiles differences are found in 31¹²⁶.

The amount of 6MM^{46, 48, 72, 94, 115, 118, 127} and different polyacetylenes of the falcarinol-group^{59, 112, 118, 128} also varies depending on genetical factors.

The difference between varieties in their concentration of 6MM is reported to depend also on the location of growth, in one location the difference between varieties were high, at another location no differences were noticed¹¹⁸.

Location

The amount of volatiles, 6MM and polyacetylenes varies depending on the location^{75, 129}.

The influence of the location is mainly an effect of climate⁷⁴. Carrots grown in a simulation of the Californian winter climate is more sweet and contain more sugar than carrots grown in a simulation of the Florida or Wisconsin summer climate⁸⁹.

Much emphasis has been used to compare carrots along a north south axis in Scandinavia^{66, 67, 70, 79, 100, 101, 130-133}. Carrots grown in northern Sweden and Finland are considered to have a higher content of sugar^{100, 130} although studies in Norway could not confirm this^{68, 70, 79, 131-133}. The content of monosaccharides are however mostly higher when carrots are grown in the north^{100, 130, 131}. This has been used to explain the observation that carrots grown in the north tastes sweeter and are crisper¹⁰⁰.

The sensory profiles from carrots grown on field and in phytotron located in the north and south of Norway are illustrated in figure 11 on the next page. The carrots grown in the north are more sweet and crisp¹⁰¹. They are also lower in colour hue and colour strength something that probably has a connection with the measured lower content of carotenoids¹⁰¹. Carrots grown in southern Norway scores higher on bitterness,^{70, 134}.

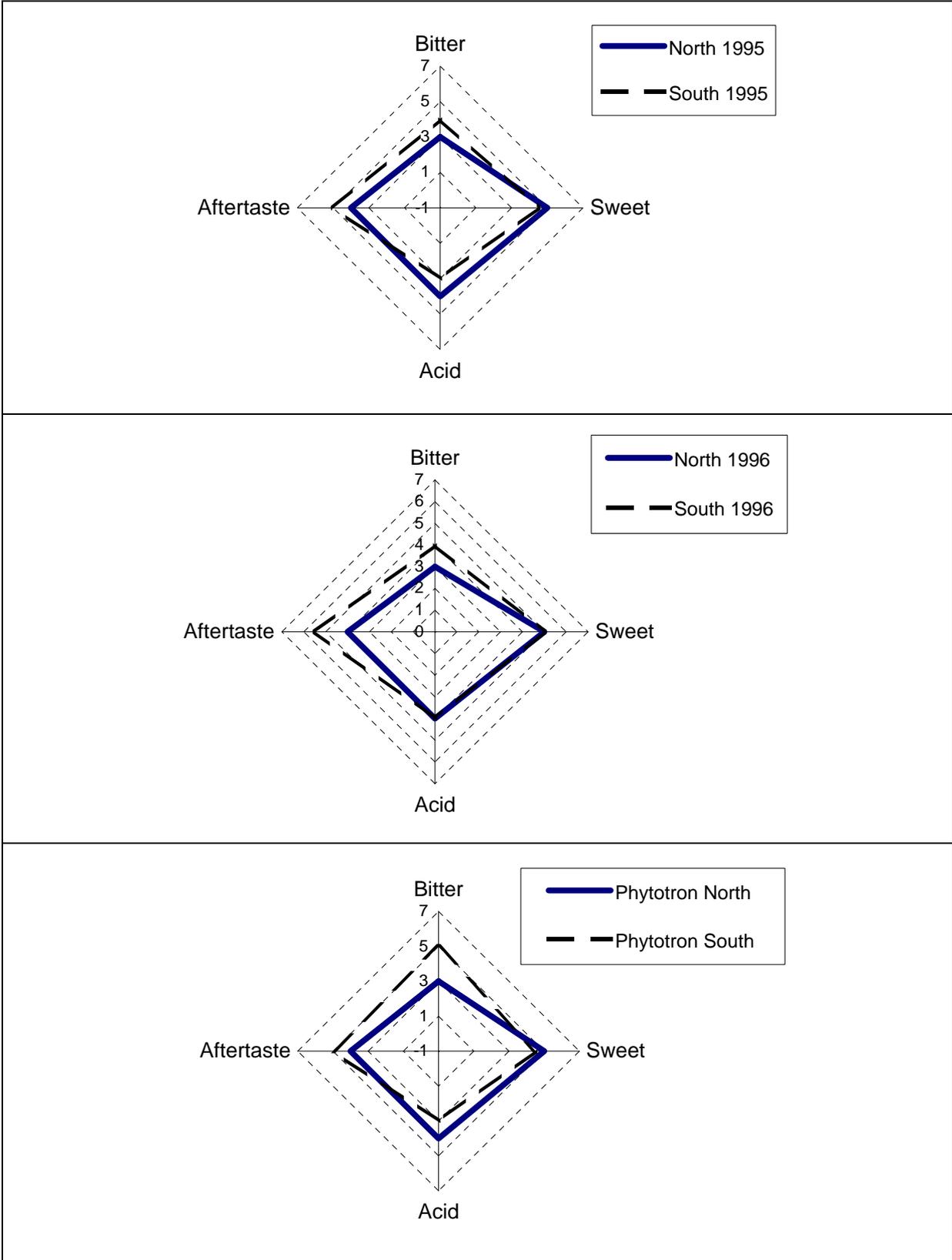


Figure 11. Comparison of sensory profiles of carrots grown at northern and southern locations. Bitter= Bitter taste, Sweet= Sweet taste, Acid= Acidic taste, Aftertaste= Aftertaste¹⁰¹.

Climate

The climate has a greater influence on chemical and sensory parameters than soil type³⁵. Light has the major influence on the morphological features of the carrot, while warmth is more important to the sensory and chemical properties⁷⁴.

Temperature

Carrot is a cool-season crop, with a minimum temperature requirement for growth on 5° C and an optimum temperature for growth between 18-25° C^{135, 136}. Carrots becomes shorter and thicker when grown in higher temperature, 18-21 °C⁷⁴. Grown at lower temperatures, 9-12 ° C, root length also increases by fusing small root granules into the storage root tip³⁵. When carrots are grown in higher temperature, or when they are kept longer in the soil, the formation of lateral root are more frequent and the carrots becomes branched⁷⁴.

Among the sensory variables; terpene flavour, green flavour, earthy flavour, bitter taste, aftertaste and firmness are more pronounced when the carrots are cultivated at high temperature, between 18-21 °C. Growing carrots at lower temperatures, 9-12 °C, has a stronger influence on acidic taste, sweet taste and juiciness⁷⁴. Carrots grown at temperatures between 9 and 12 °C taste sweeter than carrots grown at higher temperatures⁷⁴. The bitter taste is not only more pronounced when the carrots are grown in higher temperatures. The duration of the bitter taste sensation is also longer⁷⁴. Bitter taste is found to be stronger after a season low in temperature and high in precipitation⁶⁸.

The total sugar content is lower in the carrots grown in lower temperature, although the amount of monosaccharides, especially fructose, are higher⁷⁴ and the sucrose content has been positively correlated to low temperatures in June⁶⁸. Most terpenes increase with increasing temperature, while α -terpinolene decreases⁷⁴.

Light

Growing carrots in phytotrons with constant temperatures but under different seasonal light regimes reveals that higher levels of light causes an increase in many chemical and physical properties of the carrot¹³⁴. Root weight and size increased with increasing amounts of light while cylindricity and bluntness was more typical to carrots grown under short light regimes¹³⁴. The amounts of dry matter, sucrose, glucose, fructose and carotene all increased with increasing daylight¹³⁴. Due to difficulties in comparing sensory analysis made on different occasions it is hard to say something about the sensory profiles of the carrots, but bitter taste tends to be higher during low, and sweet taste higher during high levels of light¹³⁴. The variation between samples is bigger when the carrots are cultivated under more light and there is also a better correlation between the perception of sweetness and the content of monosaccharides,^{134, 137}.

Soil

There are no reports found that more systematically has investigated the importance of different soils on the sensory properties of carrots. When grown in an organic, peat, soil carrots tasted sweeter when newly harvested than carrot grown in mineral soil. After storage the carrots from the organic soil tasted more bitter¹³⁸. Carrots grown in the south of Norway showed bigger differences between soil type than carrots grown in the north¹³⁹. Carrots grown in peat are more correlated to the amount of sucrose and bitter taste, while when grown in a mineral soil they are more correlated to carotene, earthy taste and firmness¹³⁹. The soil also affects the bitter taste intensity and the amount of 6MM and polyacetylenes in the carrots,

Cultivation

Carrots grown with lower nitrogen application are found to taste more intensive, fruitier, sweeter and better and at the same time less bitter and less earthy. They have higher contents of total sugar and a higher percentage of dry matter. Fertilization with nitrogen decreases the quantity and alters the composition of the essential oils,^{140, 141}.

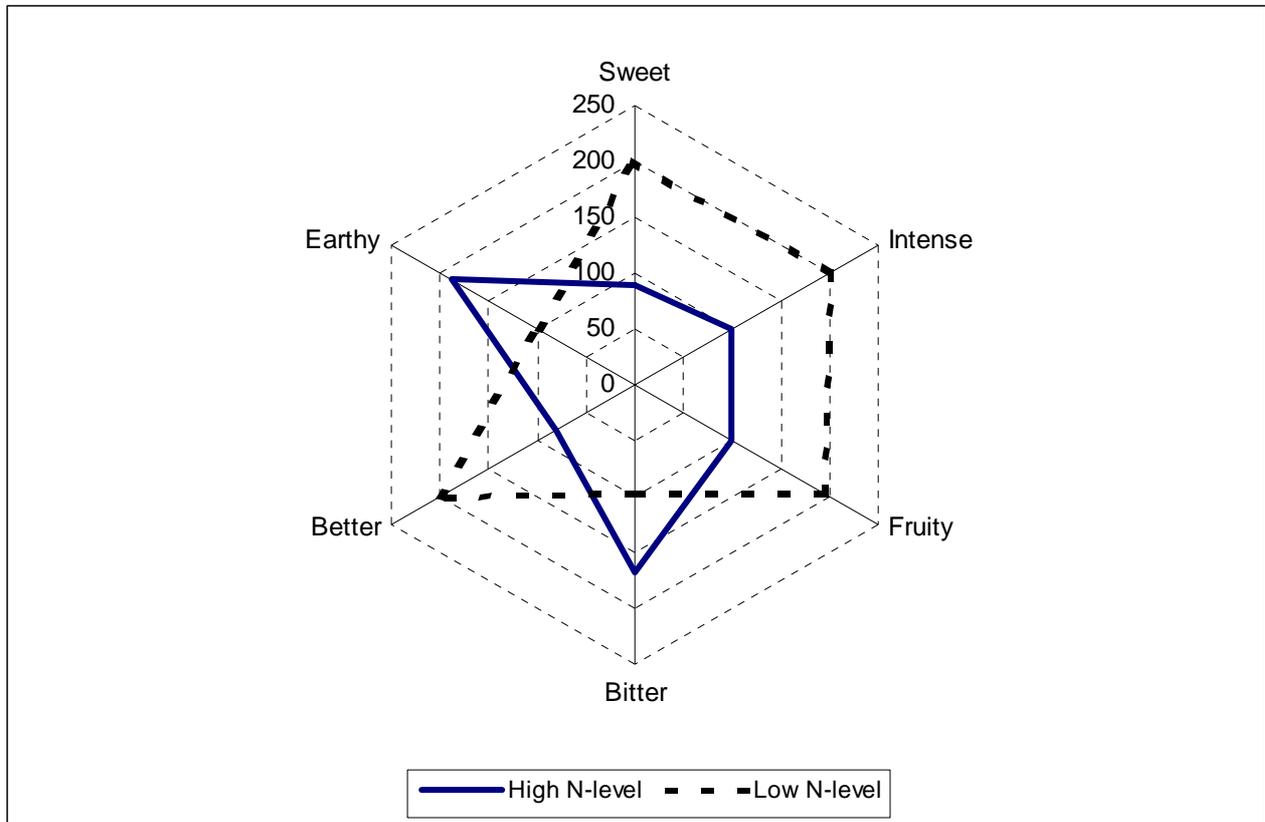


Figure 12. Taste profile of carrots grown with different levels of nitrogen-fertilisers. High N= high nitrogen level, Low N= low nitrogen level. Sweet= Sweet taste, Intense= Intensity of taste, Fruity= Fruity taste, Bitter= Bitter taste, Better= Preference of taste¹⁴⁰

Plant density has little or no effect on sensory quality or terpenoid volatiles³⁵. Delaying sowing for one to two months results in a reduction of growth and gives roots with lower dry matter content and glucose/fructose ratio but higher amounts of reducing sugars in root dry matter. The longer the growing season the higher is the amount of sucrose while the amount of monosaccharides decreased from the first harvest at 70 days to reach a constant level at about 130 days after sowing^{16, 17}.

Processing

Harsh taste in carrots often arises already in field,⁹⁹ while bitter taste is more common after storage^{85, 104, 142}.

Post harvest handling, especially storage has important impact on the properties of the carrot⁷³. Drought, water logging, frost, parasites, heavily shaking during harvest and transportation or storage under unfavourable conditions, can stress carrots. They react on stress with an increase in off-taste, for example bitter taste. Different varieties react differently upon stress⁷³.

A common reaction on stress in plants is an increase in ethylene production and a stimulation of respiration^{124, 143-147}. High concentrations of CO₂ in the air inhibit these reactions^{148, 149}.

The fact that carrots sometimes grow more bitter when attacked by pathogens^{42, 150, 151} when exposed to frost¹⁴⁵ waterstress or drought¹¹⁹, can be explained as a result of higher ethylene concentrations and higher respiration rates^{38, 98, 152}.

Higher scores for bitterness during storage are correlated to lower scores for sweetness^{38, 147, 153}. The amounts of sugar are at the same time lowered as a result of the increase in respiration rate induced by stress. Heavily shaking and storage of carrots exposed to ethylene lowers the amount of sucrose while the amount of reducing sugars remains or increases^{38, 113, 154}. The lowered sugar content probably contribute to the more detectable bitterness in the carrots during storage⁷³.

The amount of 6MM increases with temperature in ethylene enriched atmosphere⁵¹ and when stored^{47, 51, 109, 146, 155-158}. The concentration of 6MM reaches its peak a few days after the start of storage and then declines more or less rapidly^{55, 73, 156}. The concentration seldom reaches above the detection threshold^{55, 73}, but nevertheless the scores for bitter taste increases when carrots are exposed to ethylene¹¹³, heavily shaken,³⁸ or washed in a mashine¹⁵².

The concentration of terpenes is unchanged when carrots are stored in an ethylene-enriched atmosphere^{99, 113}, but decreases when heavily shaken³⁸. Refrigerated storage sometimes increases the concentration of terpenes¹²⁶, 6MM and faltarindiol¹²⁹.

Low concentration of oxygen, for example in Low Pressure Storage, prevents the effects of acetylene¹⁵⁹, but induces higher concentrations of ethanol and acetaldehydes¹⁶⁰.

The following correlation has been found when storing stressed carrots⁷³:

- There is a negative correlation between bitterness and sweetness, and between bitterness and sugar content
- There is a positive correlation between bitterness and terpenes
- There is a negative correlation between bitterness and sickingly sweet taste,
- There is a negative correlation between terpenes and sickingly sweet taste
- There is a positive correlation between ethanol and sickingly sweet taste

Steam blanching reduces the amounts of FaDOH but increases the amount of FaOH and to a certain extent also 6MM,¹¹⁸. By cutting of the peel and the green and dark parts away from the carrot the amount of faltarindiol in the carrot is reduced with 50%,⁵⁵.

The organic and biodynamic carrot

The term “organic” is used in many different ways and contexts. In this text an organic carrot refers to a carrot grown without artificial fertilizers and pesticides, in accordance with the EU-regulation 2092/91. Within this framework there is a wide variety of methods to produce an organic carrot. This makes it difficult to describe the typical properties of the “organic carrot”.

There are some reviews¹⁶¹⁻¹⁶⁵ bringing together the result from comparative studies between organic and conventional farming. The results from these reviews are summarised in table 6 on the next page.

Table 7. Comparison between conventional and organic produce¹⁶³.

Source	Woese, Lange et.al., 1995 ¹⁶¹	Worthington, 1998 ¹⁶⁵	Alföldi, Bickel et.al., 1998 ¹⁶³
Number of studies compiled	150	86	33
Covering period	1926-1993	1926-1993	1993-1998
Nitrate content	+	+	+
Vitamin content	=	+	(+)
Mineral content	=	(+)	=
Quality of protein	=	(+)	Not mentioned
Quality when processed	-	Not mentioned	=
Fodder quality	=	+	Not mentioned
Fodder preference test	+	Not mentioned	(+)
Sensory test	=	Not mentioned	(+)
	+ organic produce appears as better, (+) organic produce appears as slightly better - organic produce appears as worse		

There are only a few studies on carrots covering the difference between cultivation systems. Most of the results reported are comparisons between mineral and organic fertilizers. The results are based either on samples from field trials or on samples collected from farms or shops.

Annular variation and site-specific factors contribute more to the properties of the carrots than the fertilization system¹⁶⁶.

The dry matter content tends to be higher in organic carrots than in conventional^{167, 168}.

The concentration of nitrate is often higher in conventional carrots^{166, 169-177}. Also the amount of crude protein is usually higher in conventional carrots^{168, 169, 174, 176}. The proportion of pure protein in relation to the amount of crude protein is reported to be higher in the organic carrots^{5, 6, 174, 176, 178, 179}.

Composted farm manure in comparison to mineral fertiliser increases the amount of carotene and lowers the amount of ascorbic acid^{170, 175, 180, 181}. However the opposite effect has also been reported¹⁷³. The concentration of carotene seems to be more dependent on the amount of manure than on the type¹⁷⁷.

The amount of sugar seems to be slightly higher in organic carrots^{169, 175, 182}. This might be due to higher amounts of sucrose in the organic carrots^{177, 182}. On the other hand lower amounts of monosackarids in the organic carrots has also been reported^{169, 174, 176}. These differences can perhaps be explained by different rates in development within the two systems.

Several studies have been published from the Research Institute for Biodynamic Farming at Darmstadt in Germany. The results are compiled in table 7 as an overview of the difference between organic and conventional carrots.

Table 8. Different properties of carrots due to fertilising system. Results from different field trials at the Research Institute for biodynamic farming in Darmstadt, 1964- 1986

	Report			
Amount of	Abele, U., 1987 ¹⁷⁶	Klein, 1968 ¹⁸³	Wistinghausen, 1979 ¹⁶⁹ /Samaras, 1977 ^{184*}	Wistinghausen, 1984 ¹⁷⁴
Dry matter	+		+	=
Nitrate	-		-	-
Crude protein	-		-	-
Pure protein	-		=	+
Amino acids	-		-	
Monosaccharides	+	=	+	-
Disaccharide	-	=	+	+
Activity of enzymes	-		-*	=
Storage losses	-		-*	=

. + higher in organic farming, - lower in organic farming, = no difference

In discriminating sensory test, triangular test, the assessors could correctly point out the organic carrots^{166, 173, 185}. However the assessors could not agree on which carrots tasted the best.

The organic carrots have sometimes shown better sensory ratings¹⁸¹. This contradicts results where the organic carrots got lower ratings, mainly because of their appearance and their higher woodiness¹⁸⁶. Organic carrots are reported sweeter^{177, 182}, more bitter⁷¹ and less bitter¹⁸², than the conventional carrots. The differences in taste are more accentuated after storage¹⁸⁷.

Skilleby long term trial 1991-

When the K-experiment ended in 1990 field studies were established within Skilleby research farm. This made it possible to evaluate the consequences of different treatments within a given farm situation. The aim was to study how the farm's own manure could best be treated and used to promote soil fertility characteristics, the economic use of nutrients, the level of output and the nutrient quality of the products, while minimising negative impacts to the environment, all in the context of biodynamic agriculture^{8,9}

Crop rotation

The effects of applications of non-composted and composted manure were studied, with and without biodynamic preparation treatments, at three levels of application (12.5, 25 and 50 tons per ha 1991-1995 and 0, 25 and 50 tons per ha 1996-2008). This resulted in all together 12 treatments with 2 – 4 replications of each treatment. The trial was established on each of the five fields on Skilleby farm. A five year crop rotation was used:

1. oats with under sowing
2. ley I
3. ley II
4. ley III
5. winter wheat with the application of farmyard manure

This crop rotation was designed to improve the humus content and soil fertility¹⁸⁸.

Geographic localisation

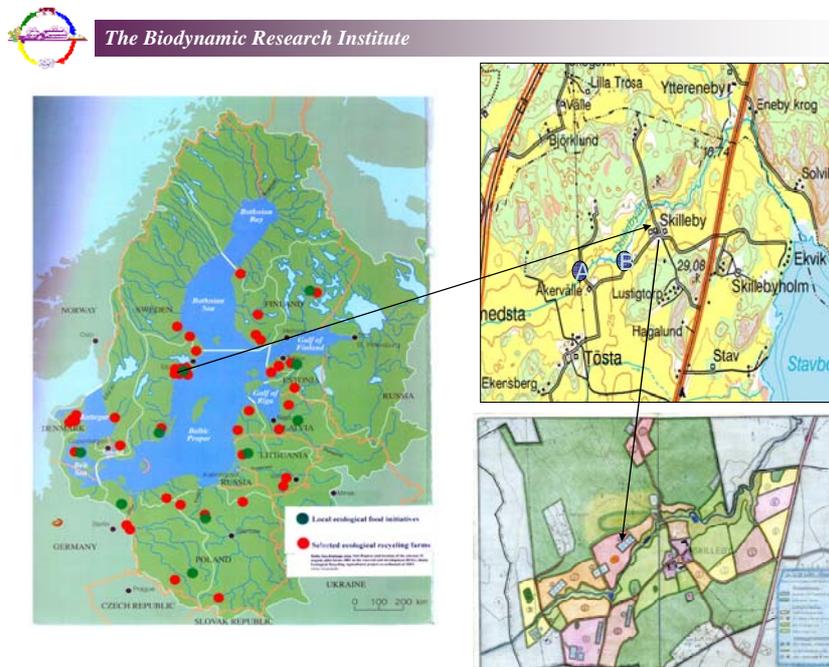


Figure 13. Localisation of the Skilleby long term trial in east Central Sweden, at latitude 59° North and longitude 18° East, 30 – 40 m above sea level.

Climate



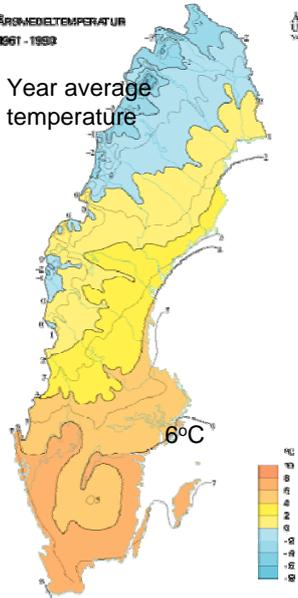
15 March 2002

dynamic Research Institute

SMHI

ÅRSMEDELTEMPERATUR
1961 - 1990

Year average
temperature



SMHI

ÅRSNEDERBÖRD
Uppskattningsvärden för
våttår 1961-1990

Year average
precipitation

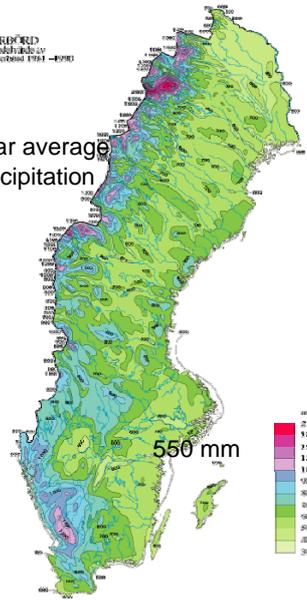


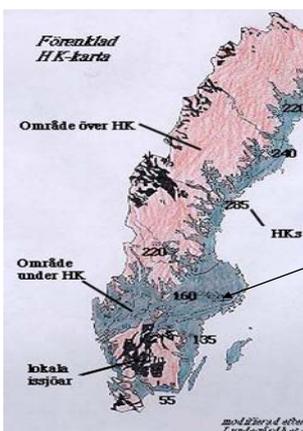
Figure 14. The annual average temperature on the Skilleby experimental farm is 6,2 °C and annual average precipitation 590 mm. The vegetation period is approximately 7 months.

Soils

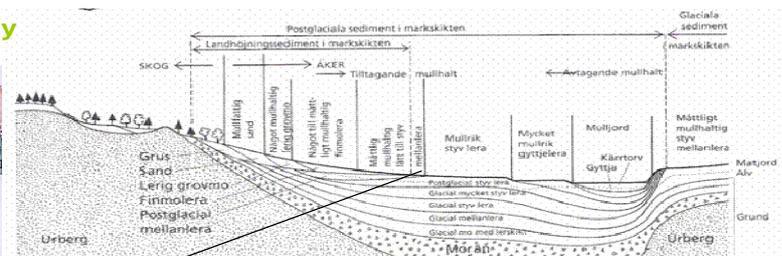


The Biodynamic Research Institute

Natural history

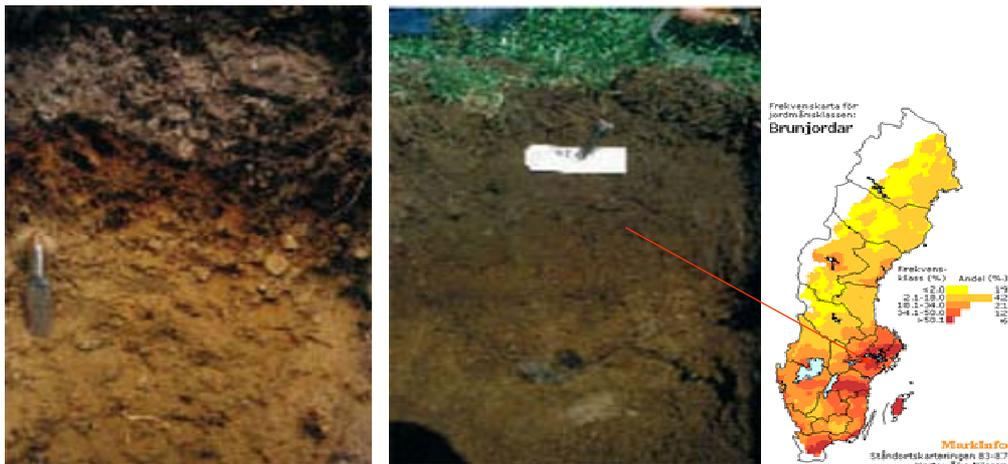


Map with simplified high coast-line (HK), Area above the HK and under the HK.



In Sweden most arable land is found where there are sedimentary soil types below the high coast-line after last ice time 10 000 years ago..

Figure 15. The soils are postglacial sedimentary clay and loam with low humus content in the lower parts mixed with some mud clay

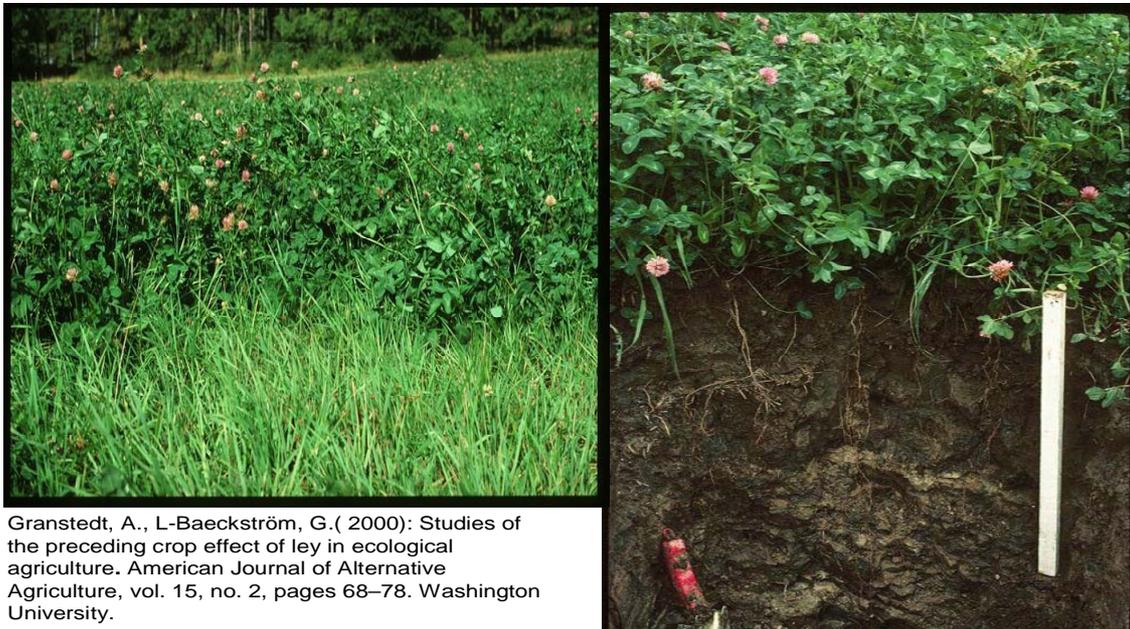


Humus-iron podzol (Soil survey archive)

Brown earth

Pictures showing main soil profile types in Sweden. The ongoing leaching in podzol soils results in the characteristic layer of bleached soil. Brown soils is result of a more strong reverse process trough basic nutrient components uptake of the vegetation and fast decomposition on surface.

Figure 16. The soil formation after the glacial time is depending of the mineral parent material, climate, topography and the natural vegetation followed of the rather late start of cultivation about 500 – 1000 years ago on this actual soils.



Granstedt, A., L-Baeckström, G.(2000): Studies of the preceding crop effect of ley in ecological agriculture. American Journal of Alternative Agriculture, vol. 15, no. 2, pages 68–78. Washington University.

Figure 17. The focus within the Skilleby long term trial is to study how soil fertility and food quality is effected by manuring regimes and soil treatments. Between 1991 and 1996 a special study comparing the effects on different durations of ley and the effects of the preceding crop¹⁸⁹.

Design of field trial



The Biodynamic Research Institute

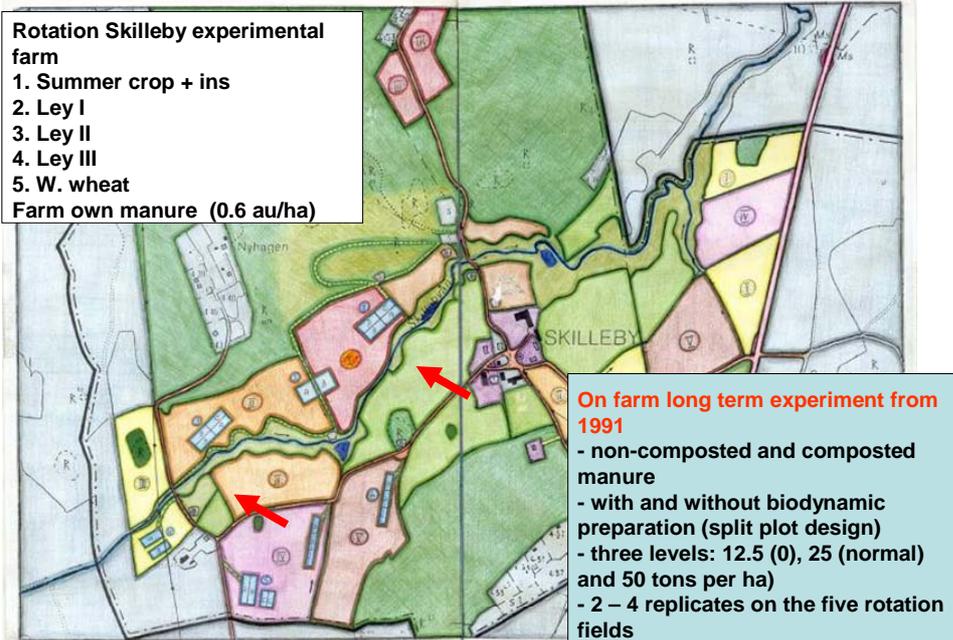


Figure 18. The field trials are located on representative spots in each field with start in the winter wheat autumn 1991 on field number one. The following year winter wheat was sown 1992 on field number 2 and so on until 1995 when the trial plots were established on the last field, number 5.



The Biodynamic Research Institute

Skilleby long-term trial started in 1991 and still continuing



Experimental plan from 1991

Main plot	Treatments winter wheat
F1	Not composted manure 12.5 ton (0 from 1995)
F2	25 ton
F3	50 ton
K1	Composted manure 12.5 ton (0 from 1995)
K2	25 ton
K3	50 ton
Subplots +	BD preparation each plot each year
-	Without BD preparation

Figure 19. Field trial implementation and the experiment design.

During 2006, 2007 and 2008 the plots F1 were fertilised with pelleted chicken manure in the field trial where carrots were cultivated. The amount of chicken manure corresponded to F3 and K3 concerning the amount of available nitrogen. The chicken manure was only applied in the part of the plot where carrots were cultivated.

Weather conditions

Data on temperature and precipitation was obtained from the Swedish Meteorological and Hydrological Institute weather stations in the proximity (within 20 km), from the field trial and from 2004 and onwards on a climatic station located on the farm (figures 8- 14).

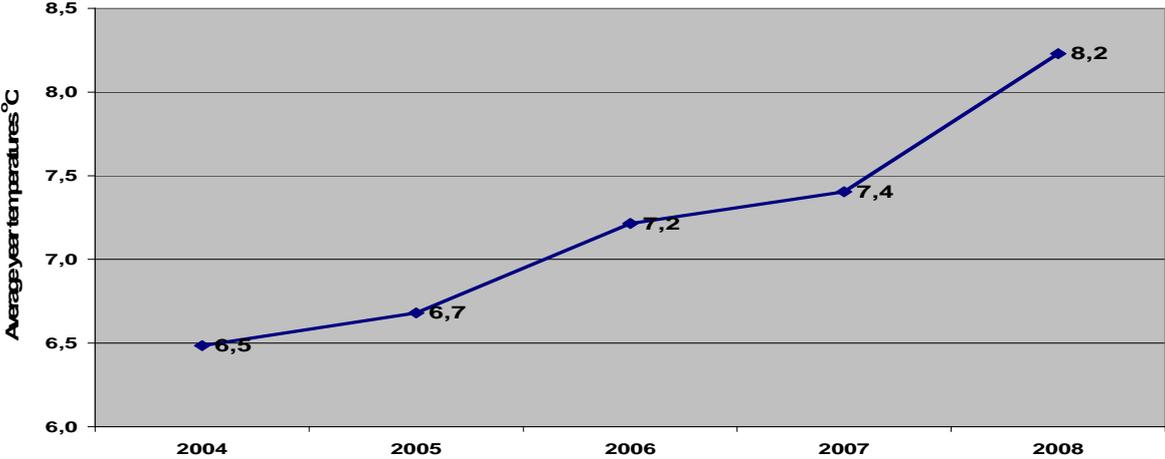


Figure 20. Average annual temperatures during 2004 – 2008.

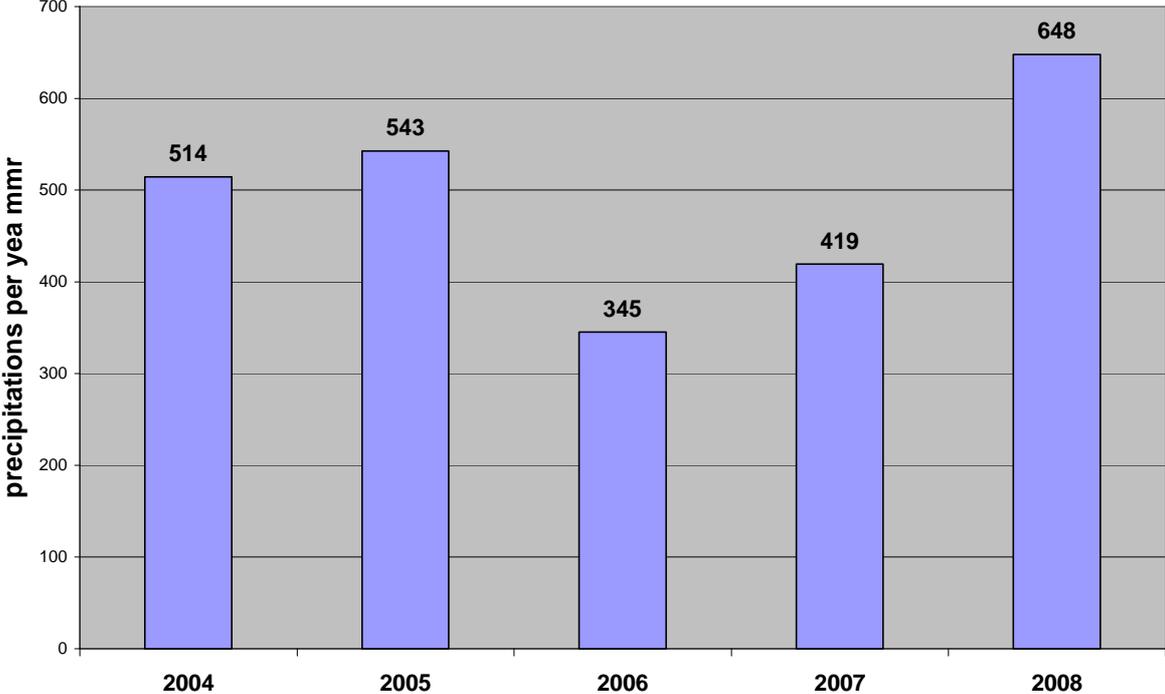


Figure 21. Average annual precipitation during 2004 – 2008

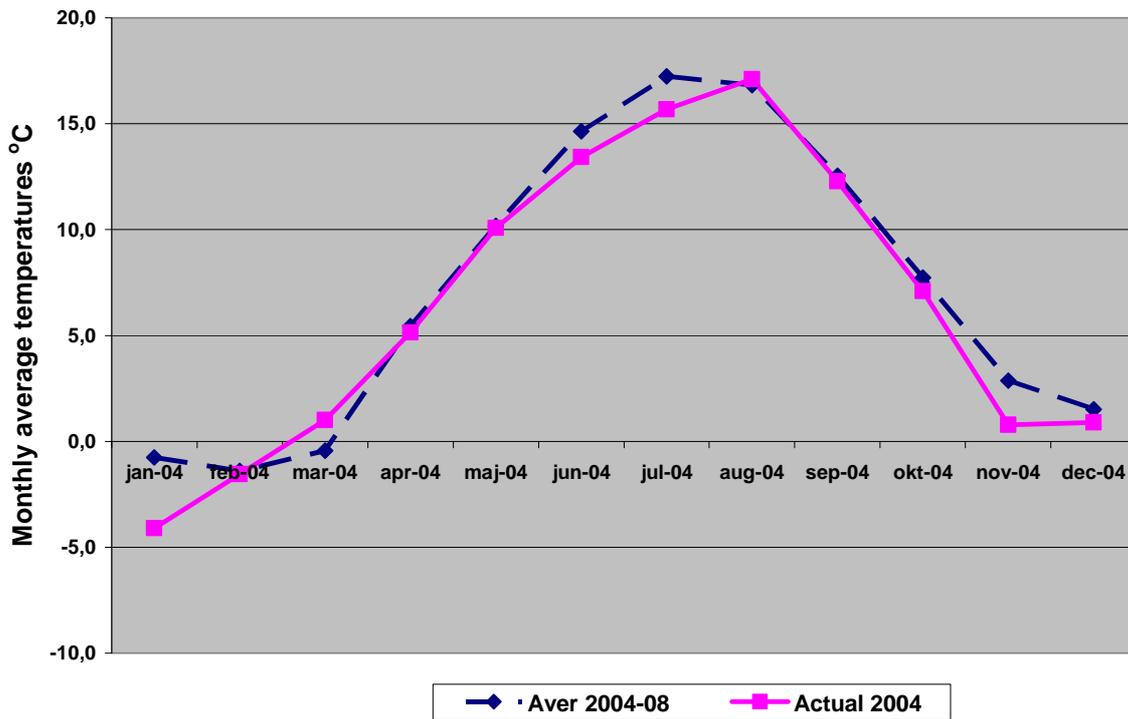


Figure 22. Monthly average temperatures for 2004 (actual) compared to the average monthly temperatures during the five years period 2004 – 2008.

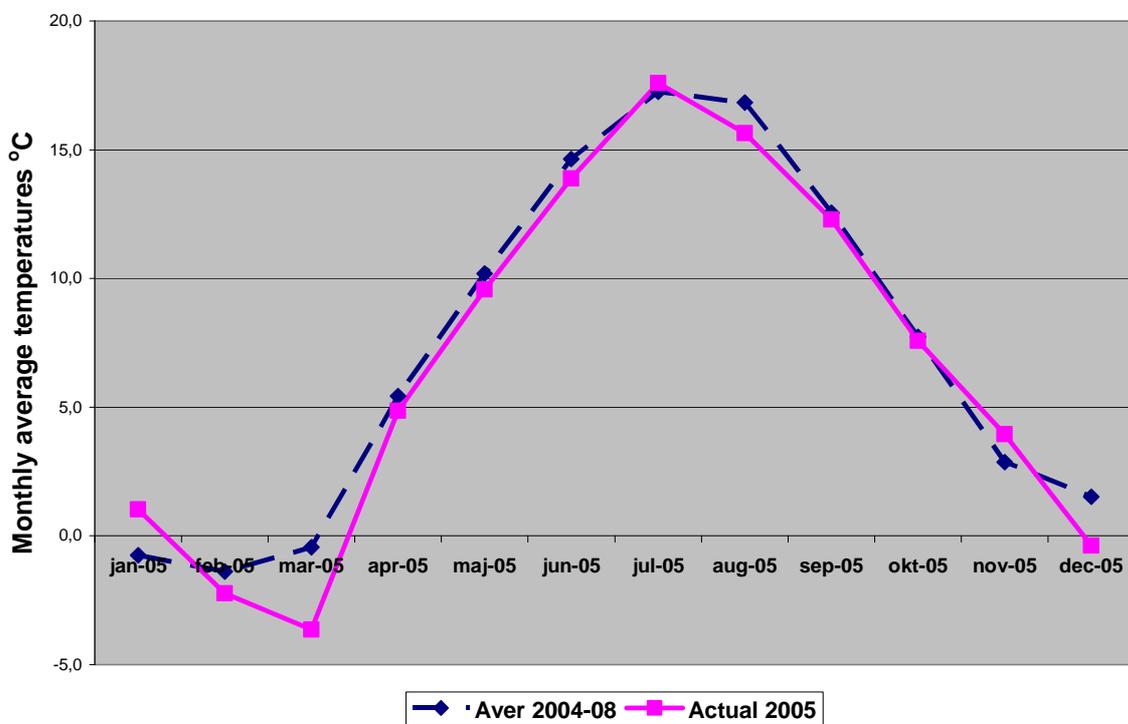


Figure 23. Monthly average temperatures for 2005 (actual) compared to the average monthly temperatures during the five years period 2004 – 2008.

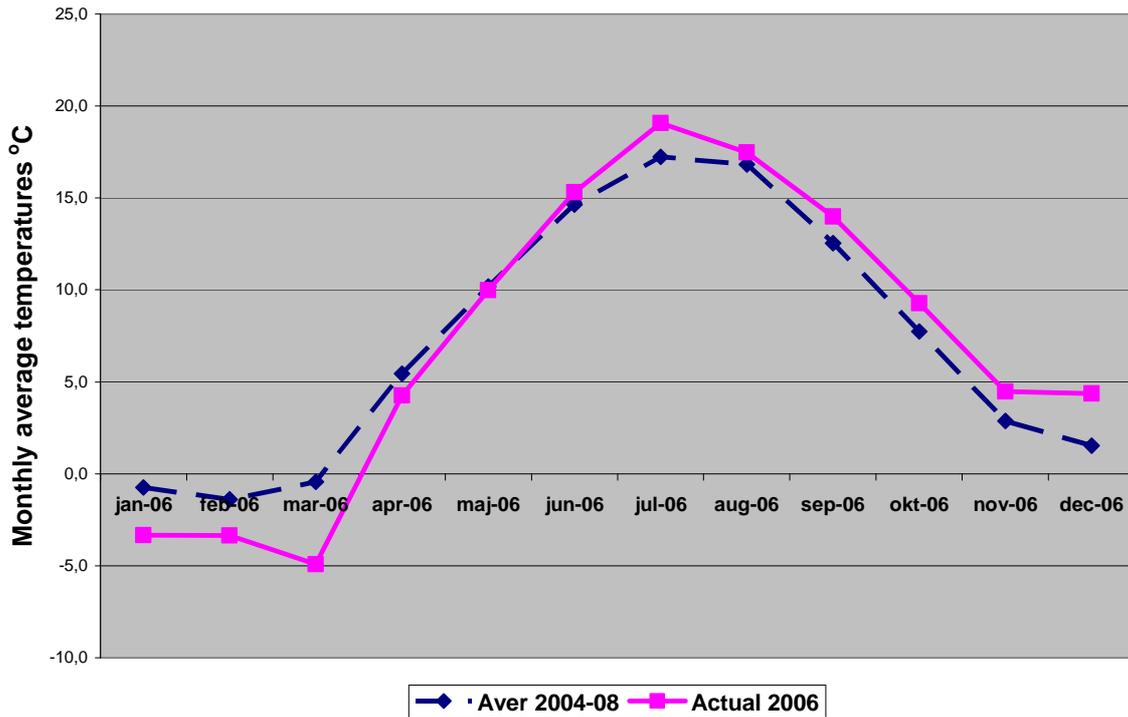


Figure 24. Monthly average temperatures for 2006 (actual) compared to the average monthly temperatures during the five years period 2004 – 2008.

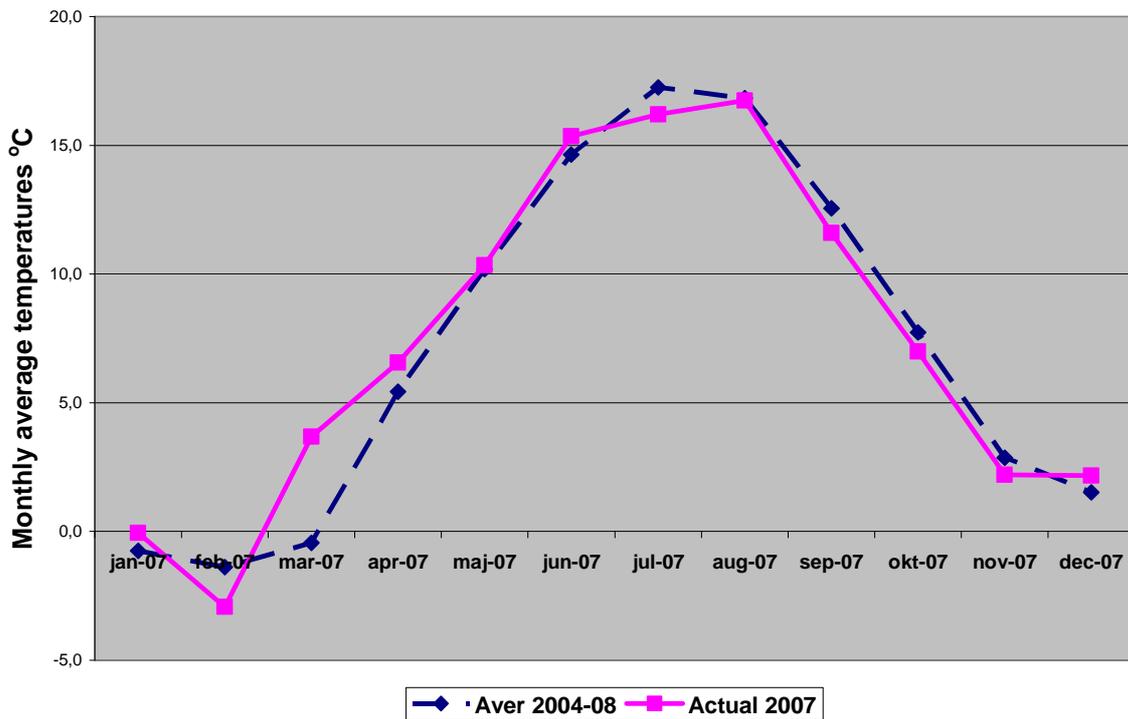


Figure 25. Monthly average temperatures for 2007 (actual) compared to the average monthly temperatures during the five years period 2004 – 2008.

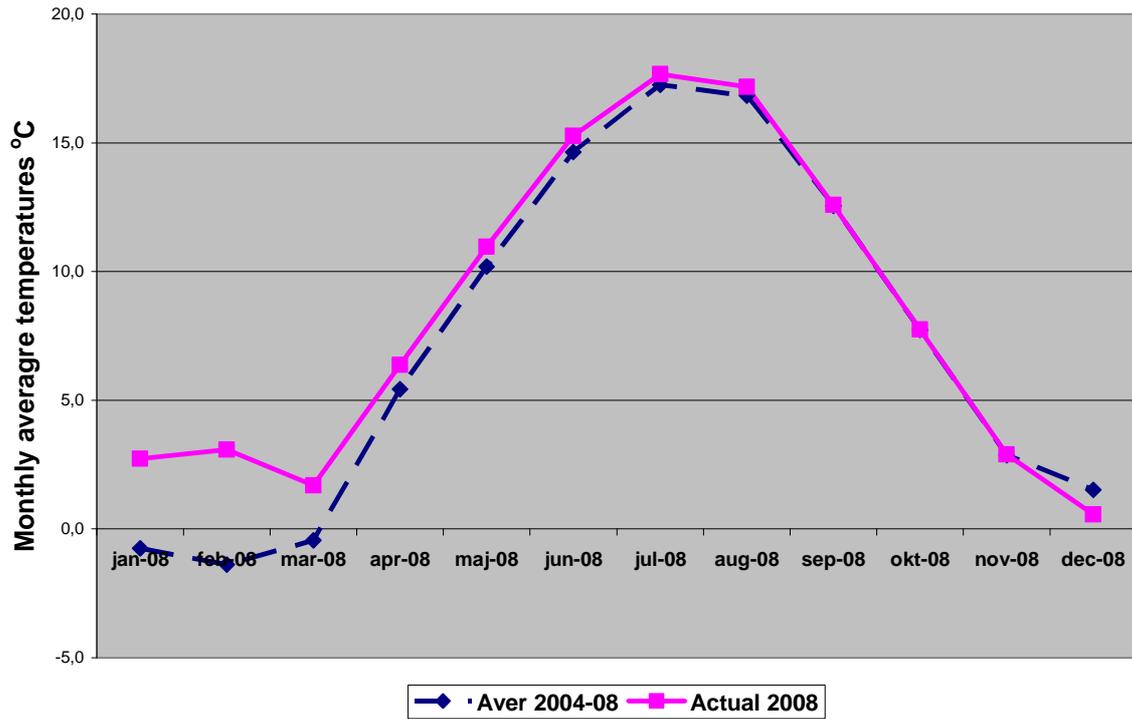


Figure 26. Monthly average temperatures for 2008 (actual) compared to the average monthly temperatures during the five years period 2004 – 2008.

Material and Methods

Soil

Total carbon and nitrogen contents were measured with LECO CHN 600 (elemental analysis) and available P, K, Ca, Mg, Na were analysed after extraction in ammonium lactate (AL) solution (SS-ISO 11464 and SS 028310).

Carrots

Morphology

The shape of the carrot roots were determined according to methods developed by Bleasdale and Thompson¹⁹⁰, Snee¹⁹¹ modified by Rosenfeld⁷⁴. On 15 roots per sample weight and length of the root was determined together with maximum diameter and thickness at each fifth and at 9/10th along the length of the root counted from the root top. The roundness of the root tip was calculated by dividing the root diameter at 9/10th of the root length with the diameter at 8/10th of the root length. The cylindricity of the root was determined by the formula $C = 1 / (\text{Max diam} / \text{Diam } 8)$, where C is the cylindricity, Max. diameter is the largest diameter along the root and Diam 8 is the diameter at 8/10th along the root seen from the root top.

Sugars

Analysis of fructose, glucose and sucrose was performed by extracting 300 mg of each sample with 4,0 ml 70% ethyl alcohol. The extracts were kept for 14 days in -20°C before further analysis. After centrifugation with a Hettich Universal 30RF centrifuge at 10 000 rpm for two minutes 1 ml of the extract was filled into glass vials and then analysed by HPLC, Prime for Windows, equipped with an R14 IR-detector, using a Asahipac Shodex NH2P-50 4E 4,6*250 columna. The eluents were acetonitrile/water 70/30 in a continuous loop with a flow of 1 ml/minute. Data were evaluated by HPLC technology Ltd, UK, Prime for Windows, PW-500. The integrated area from the samples was compared with that from external standards for fructose, glucose and sucrose. All reagents used in the analysis were of HPLC-grade, the water was of Ultra-Pore quality.

Polyacetylenes

Analysis of polyacetylenes was performed with HPLC, Agilent 1100-system (Agilent Technology) equipped with a diode-array detector using a method developed by Christensen and Kreutzmann¹⁹² with modifications. 200 mg of carrot powder was extracted with 7 ml of ethyl acetate containing 0,474% 4-chlorobenzophenone (Alfa Aesar GmbH&Co) as an internal standard. The extracts were shaken in an orbital shaker (Forma Scientific Inc. Marietta, USA) for 16 hours in darkness at 4°C. They were then centrifuged at 4 000 rpm for 5 minutes using a Hettich Universal 30RF centrifuge. 4ml of the extract was evaporated using pure nitrogen gas. The remaining solids were solved in 200µl acetone and filled into glass vials for HPLC-analysis using a Phenomenex Luna C18(2) 100*3 3µm columna. The flow was 1 ml/minute. Acetonitrile and water was used as eluents. The binary gradient as follows expressed in the percentage of Acetonitrile (AcN): 0-5 minutes steady at 20%, 5-10 minutes rising to 52,5%, 10 to 31 minutes steady at 52,5%, 31-55 minutes rising to 95%, 60 to 61 minutes falling to 20%, 61 to 65 steady at 20%.

Free amino acids

The amount of free amino acids has been determined with fine-grinded plant matter in a water-extract. The Sørensen's formal-method with titration to pH 8,5 has been used. The

method shows the amount of the amino acids that are not attached to the protein. Low values are favourable.

Extract decomposition

Determination of the changes in the electrical conductance was measured at 20°C in a water-extract of carrot tissue 1:10. The extract was then kept in this temperature and measured on a daily basis in the same way, until the conductance no longer changes. The method reflects first, the speed in the enzymatical and then the bacterial decomposition of the extract. Low values are favourable.

Total amounts of sugar

The total amount of sugar is determined using a refractometer. High values are favourable.

Quality indices according to Pettersson

Quality-indices, according to Pettersson, are an integrated value of the results from the following separate analyses with the ratio-value in parenthesis.

Free amino acids, N, mg/100 g dm (dry matter)	(375)
Total sugar content, %	(9)
Extract decomposition, Rd/Ro	(16)

The obtained value from a separate method is expressed in % of the equivalent ratio-value. The values for free amino acids and extract decomposition are "inverted" arithmetically around the value 100. The three values are added and the sum is divided by 3. The obtained value is described as the quality indices. High values-values are positive traits.

The indices are thus calculated according the formula: $((\text{Total sugar content} \cdot 100 / 10) + (200 - (\text{amount of free amino acids} \cdot 100 / 375)) + (200 - (\text{rate of extract decomposition} \cdot 100 / 18))) / 3$

Sensory test

Sensory test was performed by a trained panel consisting of 3 persons. Each sample was tested by all the panellists. The impression was formulated individually, written down and the notations were categorized. For the evaluation of the taste sensation the categories; woody, juicy, sweet, bitter and harsh were used.

Calculations

Data were evaluated by Chemstation A09.03 software (Agilent Technology). The different polyacetylenes were identified using retention time and data from the UV-spectra at 205,5 nm. The obtained spectra were compared with those given in the literature^{116, 118-120, 192}. All reagents used in the analysis were of HPLC-grade, the water was of Ultra-Pore quality.

HPLC results are based on three replicate samples. Calculations and statistical evaluations were made using the computer programmes SPSS 16.0 (SPSS Inc.). Confidence intervals and significant levels were determined by LSD and Duncan test for <0.05 using One-way Anova. To investigate relationships between climatic, morphological and biochemical data Pearson correlation coefficients were calculated.

Picture forming methods

Three different picture forming methods were used; copper chloride crystallisation, capillary dynamolysis and round paper chromatogram. The methods are described in connection with the presentations of the results.

Results

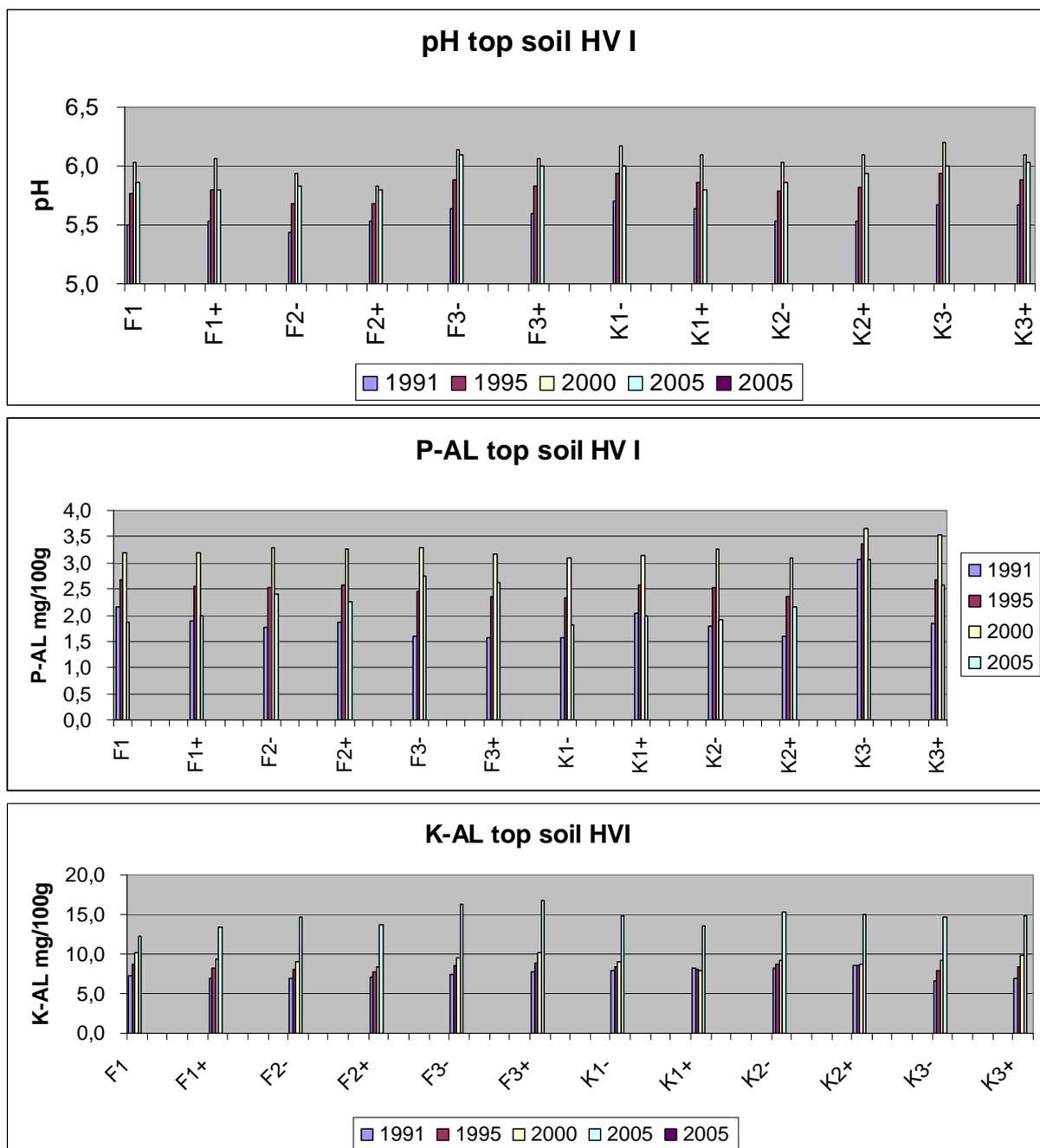
The results are divided into three parts; soils, crops in the regular crop rotation and carrots.

Soils

The soils were analysed concerning their chemical and biological properties.

Chemical properties

HV 1 The soluble P content in the soil is very low (P class 1 to P class 2) but soluble K is on a good level (K class 3). During the 15 years period from 1991 to 2000 pH, P-AL, K-AL, Mg and Ca have increased in all manured treatments (Figure 15 a-e). No differences could be observed comparing composted and not composted manure or treatment with or without BD-preparations (**Figure 27**).



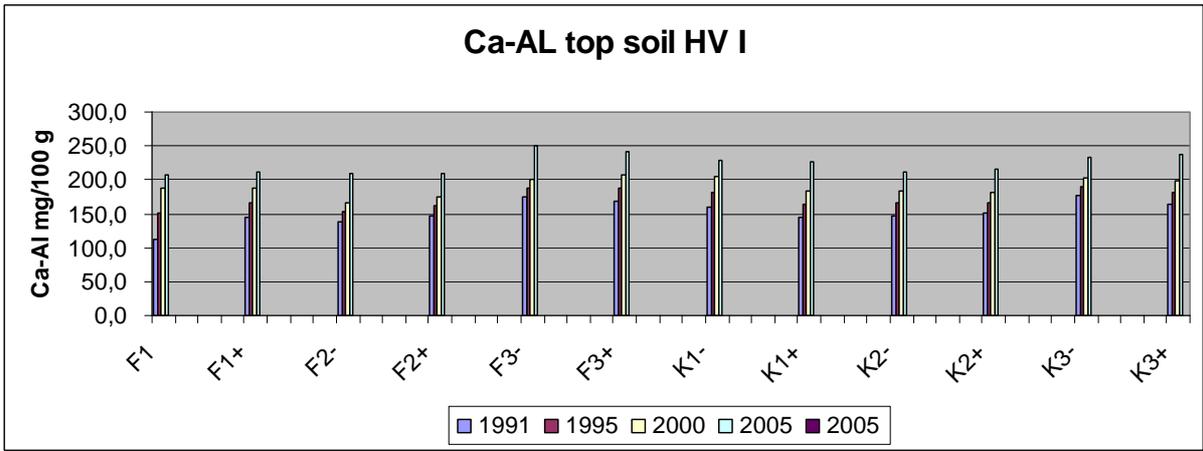
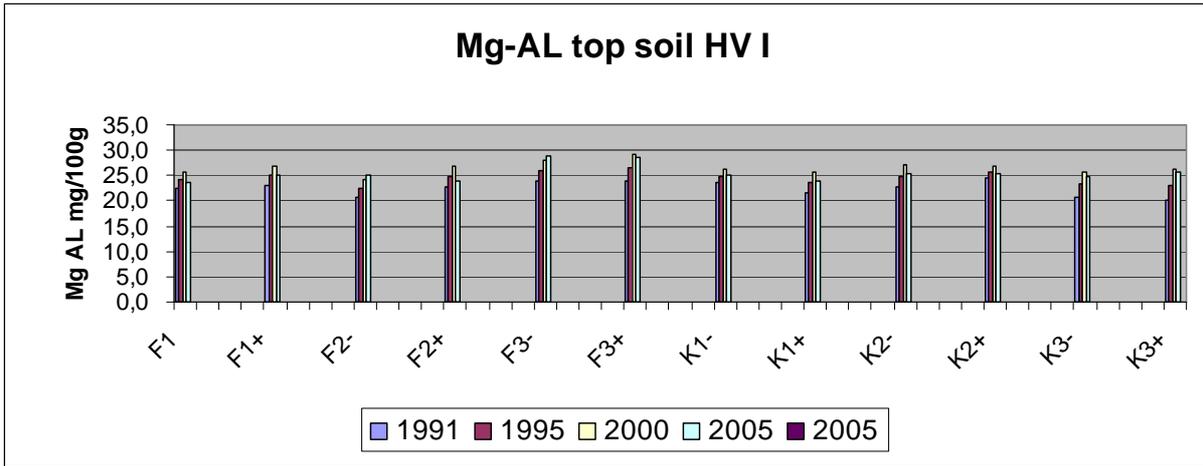
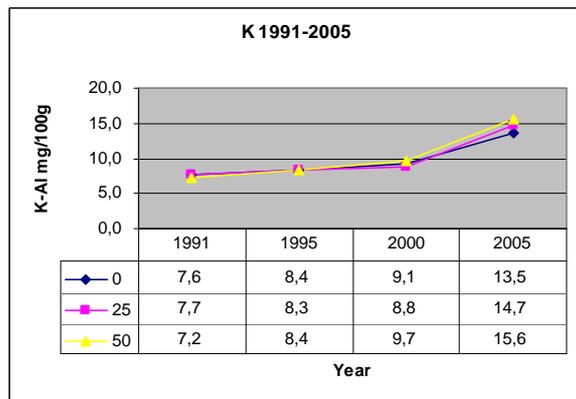
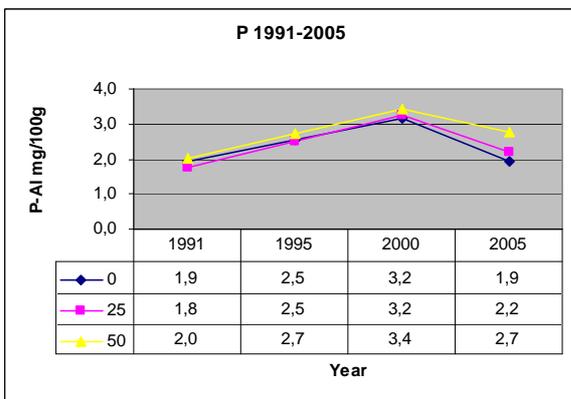


Figure 27. Soil analysis 1991, 1995, 2000 and 2005 field trial HV1.

During the period 2000-2005 a decrease of P-AL and a continuing increase of K-AL and Ca-AL was observed in all three levels of manure (Figure 27). No differences could be observed when comparing composted and not composted manure or treatment with or without BD preparations.



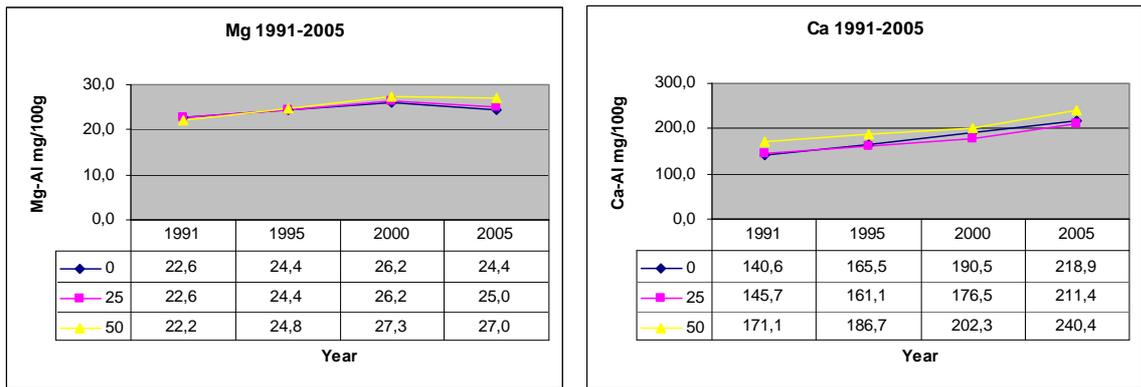
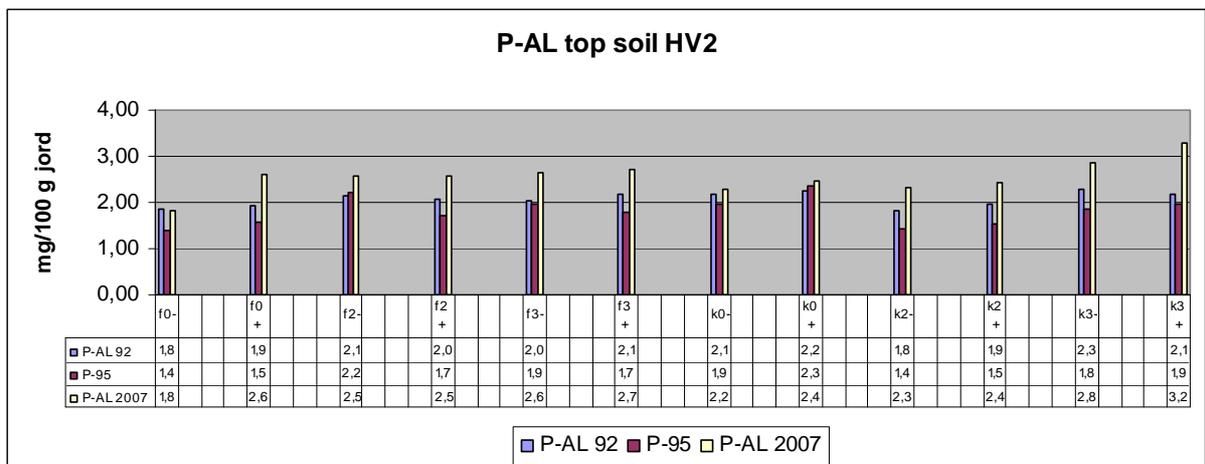
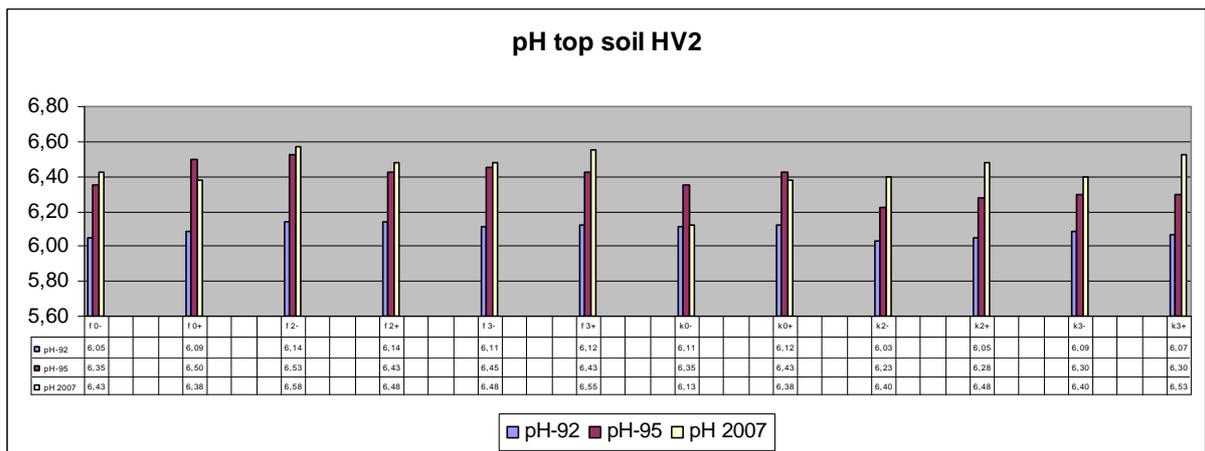


Figure 28. Soil analysis of P, K, Mg and Ca in the three manure levels (0, 25 and 50 tons per ha) before manure application to winter wheat 1991, 1995, 2000 and 2005.

HV2

On this field it was a continuous increase of all the measured chemical soil properties despite calcium during the period 2002 -2007 and with a tendency of higher values for the higher manure level and lower for the 0 manure plot (Figure 29).



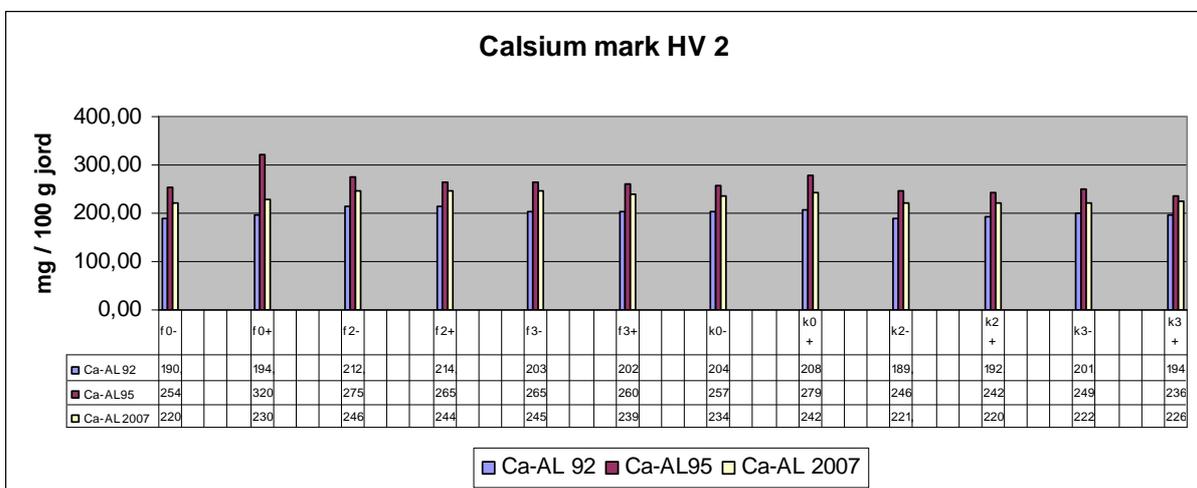
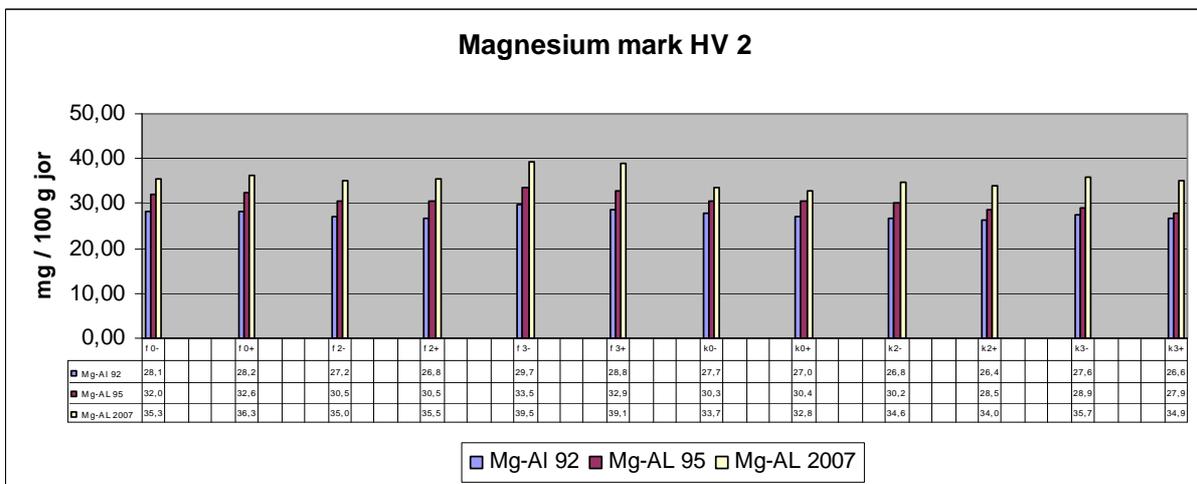
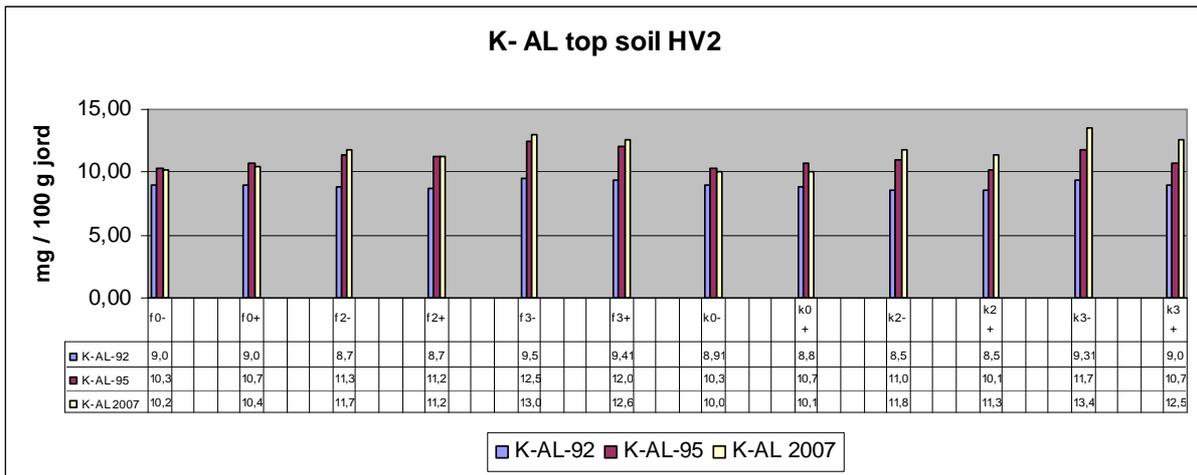


Figure 29. Soil analysis 1992, 1995 and 2007 field trial HV2 long term experiment Skilleby

Carbon content in soil

The average total carbon content in top soil increased in all treatments during the 14 years periods in HV 1 from 1991 to 2005, in HV2 from 1992 to 2006, in HV3 from 1993- 2007, in HV4 from 1994 to 2008 and in HV 5 during the 5 years 2002 – 2007 (**Figure 30**). But it was also observed variations between the treatments.

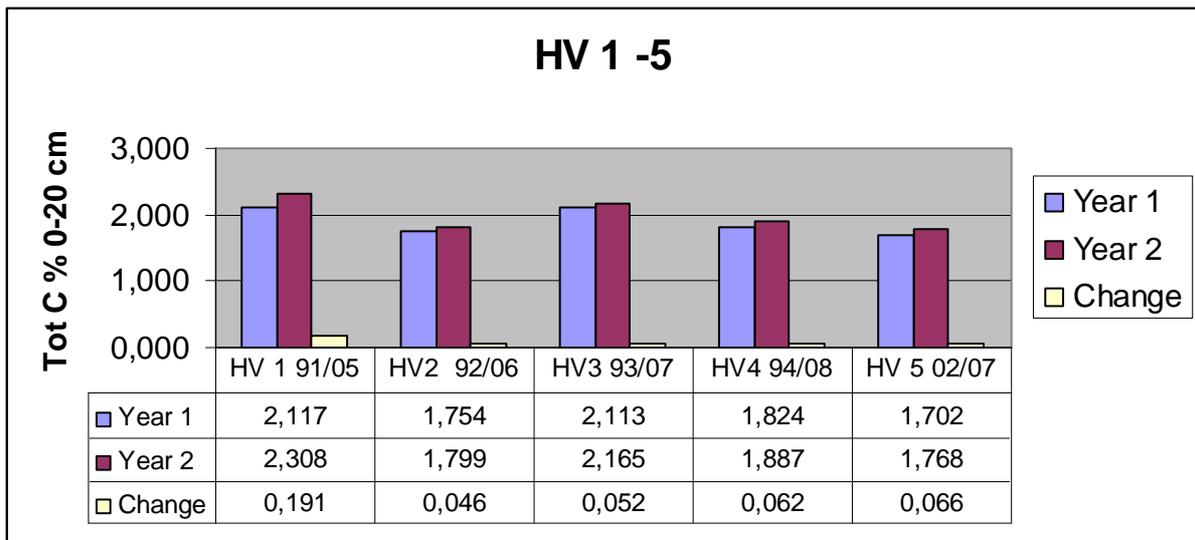


Figure 30. The average total carbon content in the top soil and average increase during the study period 1991 – 2009 measured in HV1, HV2, HV3, HV4 and HV5.

The highest carbon content was measured in field trial HV1 and HV 3. In HV 1 was also the highest average increases during the study period (**figure 30**). The total carbon content in the soil increased in all treatments in HV1 from 1991 to 2005 (**figure 31**) in average from 2.12 to 2.31 % (**figure 32**).

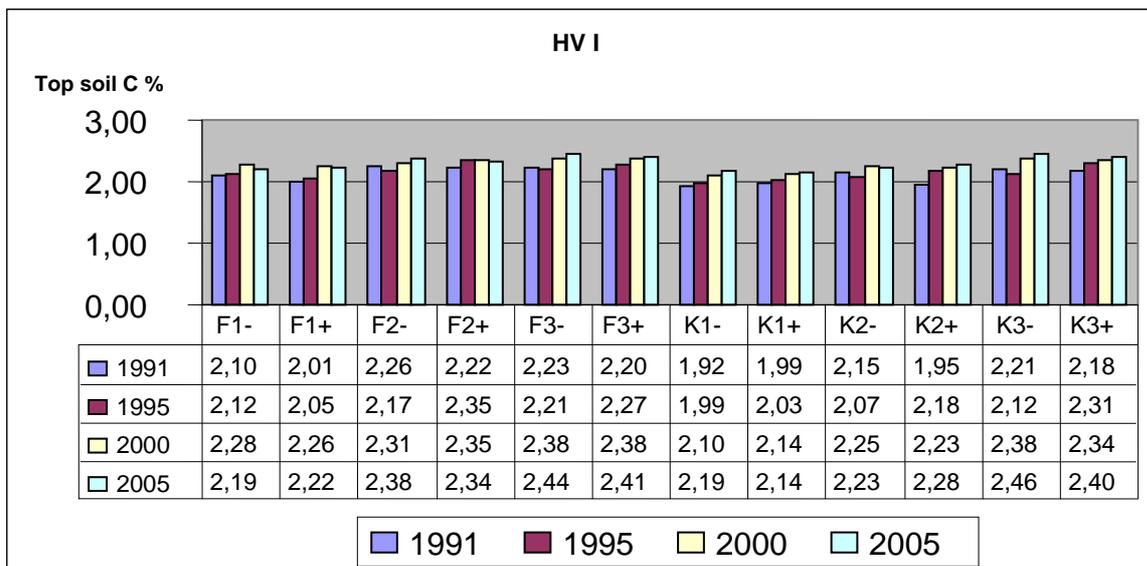


Figure 31. Total carbon content in the top soil in the 12 different treatments 1991, 1995, 2000 and 2005.

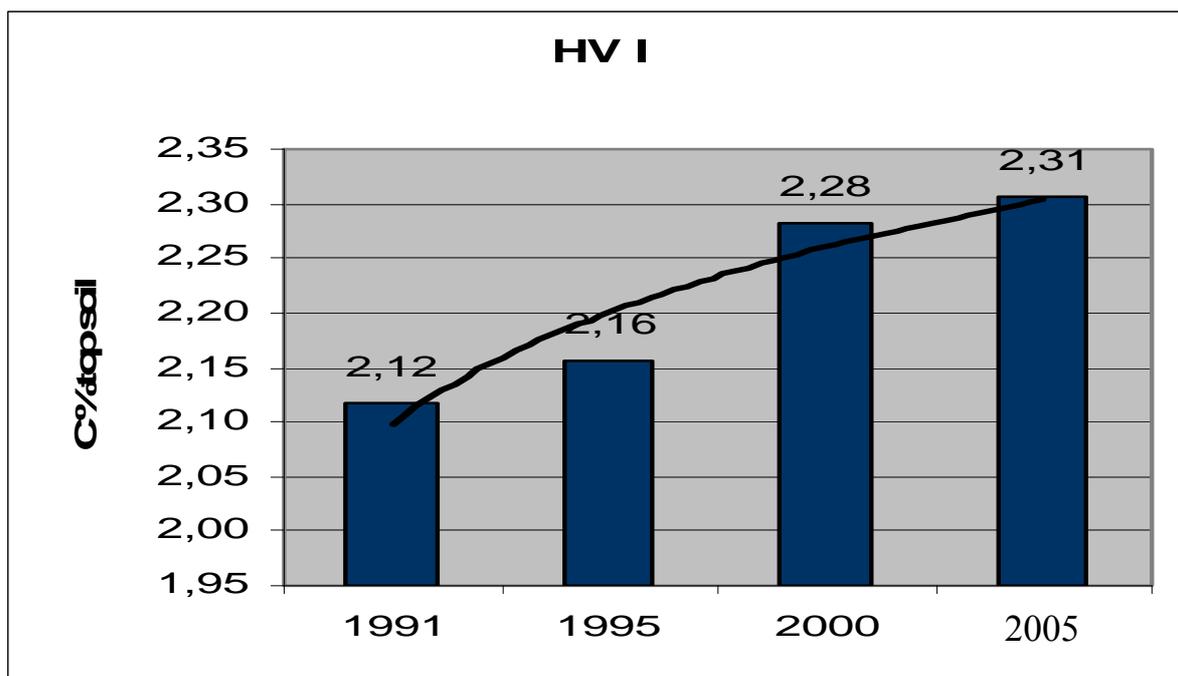


Figure 32. Average total carbon in top soil all treatments in HV 1 1991, 1995, 2000 and 2005. General trend.

The change of carbon and humus content (C % units) is in this study evaluated through calculation of the difference of total carbon content before and after the study years.

In HV 2 there was an average increase of topsoil carbon observed during the years between 1992 and 2006 from 1,75 to 1,80. There was a lower or no increase in the plots not manured after 1997 (F1 and K1) (**Figure 33**).

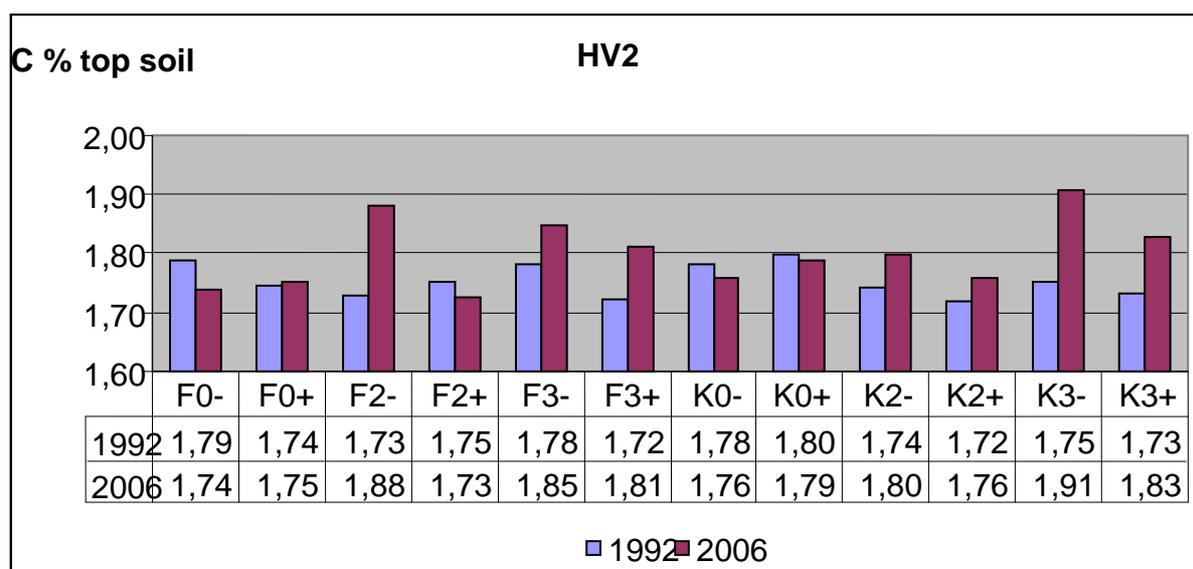


Figure 33. Total carbon content in the top soil in the 12 different treatments, 1992 and 2006 in HV 2.

The total carbon content in soil increased in most treatments in HV 3 (**figure 34**). The increase was in average from 2,11 to 2,16 during 1993 to 2007. It was impossible to see consequent differences between the treatments.

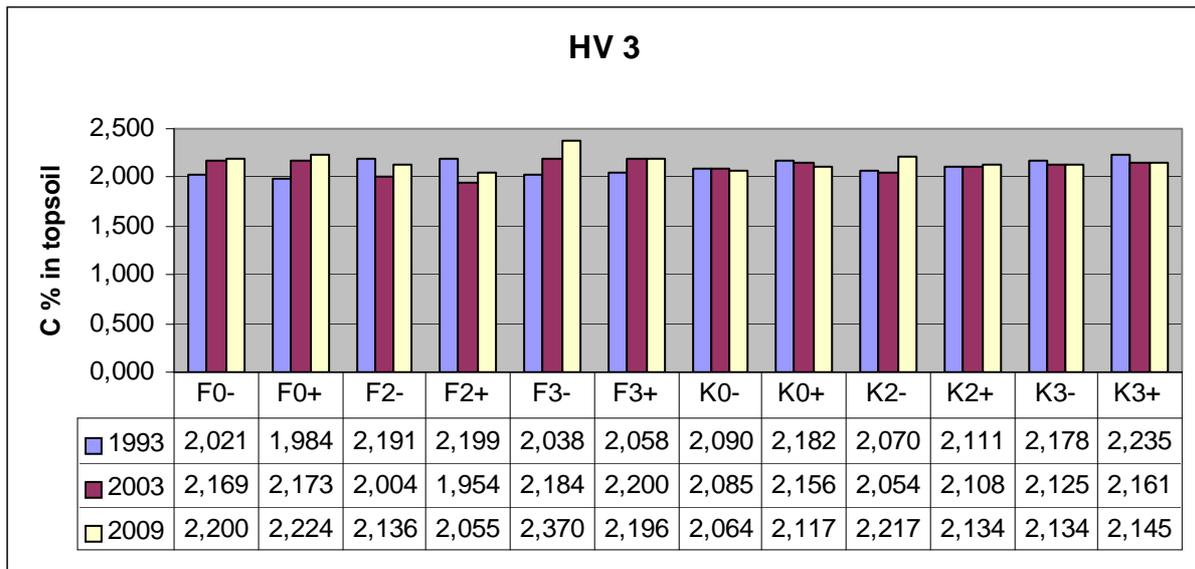


Figure 34. The average total carbon content in the top soil in the 12 different treatments 1993, 2003 and 2009 in HV 3.

In HV 4 the carbon content in the top soil increased in most treatments with the exception of the unmanured treatments (**figure 35**). The average carbon content increased from 1, 82 to 1,89 %.

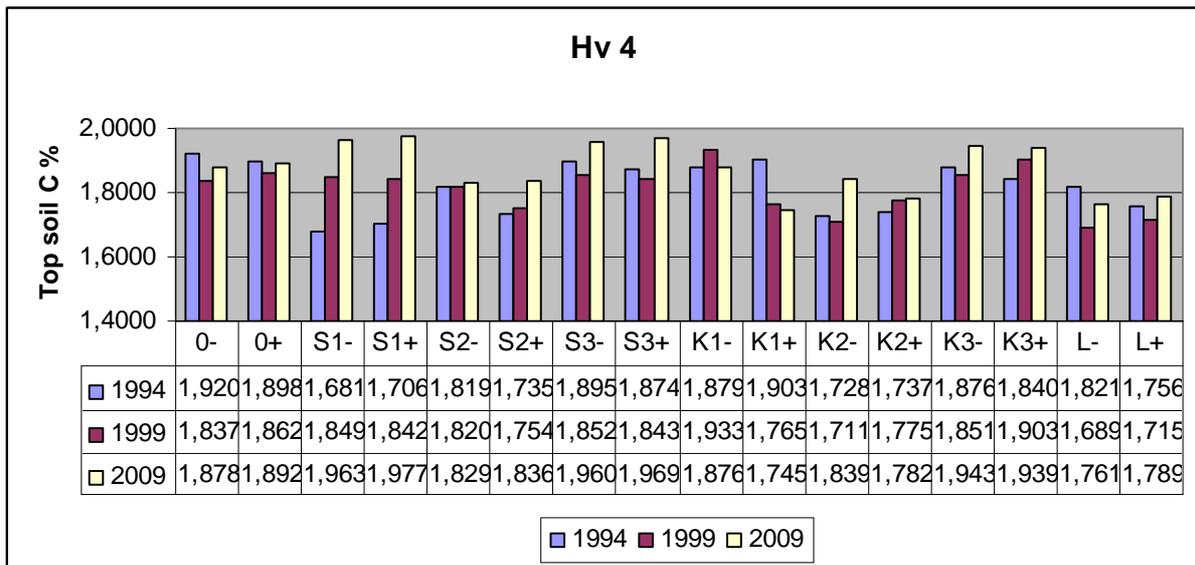


Figure 35. Average total carbon content in the top soil in the 12 different treatments 1994, 1999 and 2009 in HV4.

Also in HV 5 the total carbon content in soil increased in most treatments with the exception of the unmanured treatments (**figure 36**) in average from 1,70 to 1,77 during the five years period from 2002 to 2007.

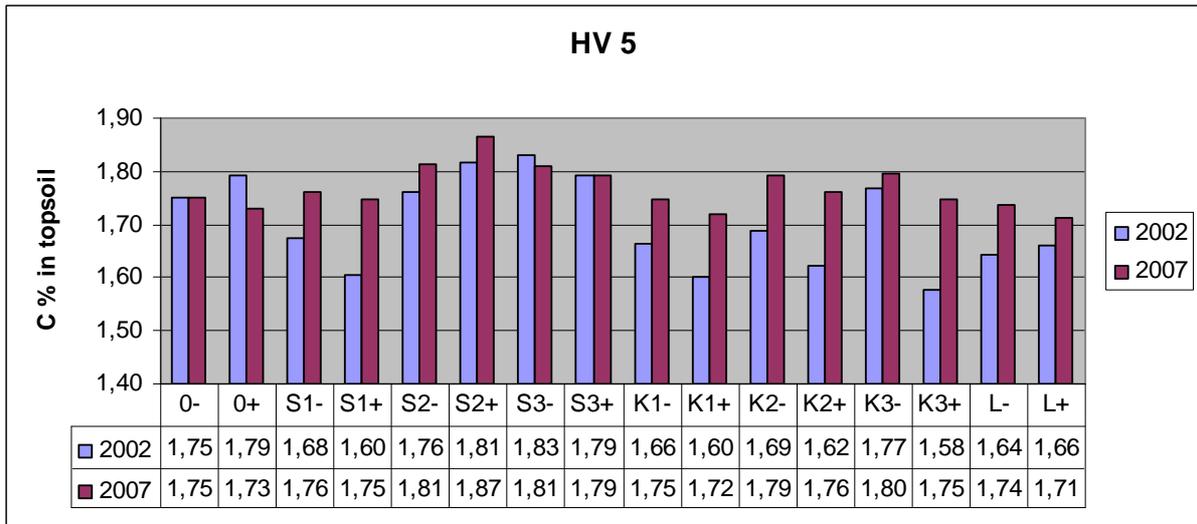


Figure 36. The average total carbon content in the top soil in the 12 different treatments 2002 and 2007 in HV 5.

Influence of manure application

The influence of manure application on total carbon and humus content is possible to observe by comparing the change of total carbon in top soil between the different treatments (**figures 37 and 38**). In HV 2, HV 4 and HV 5 it was a decrease of the carbon content in topsoil in the unmanured treatments (**figure 37**) compared to the manured treatments (**figure 38**). The average carbon content in soil was higher in the treatment using farm normal fertilising compared with no use of manure in this crop rotation. In the treatments with high manure levels were as average the carbon and humus content significantly higher (104 % higher).

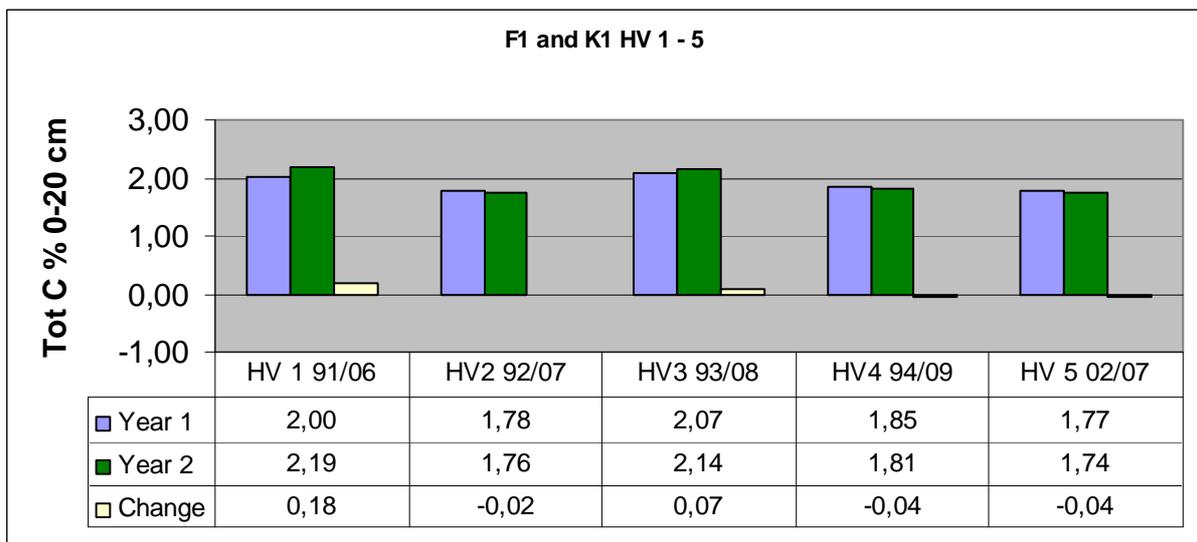


Figure 37. Total carbon content and changes in the top soils after three crop rotations without manure in HV 1 – HV4 and one crop rotation in HV 5.

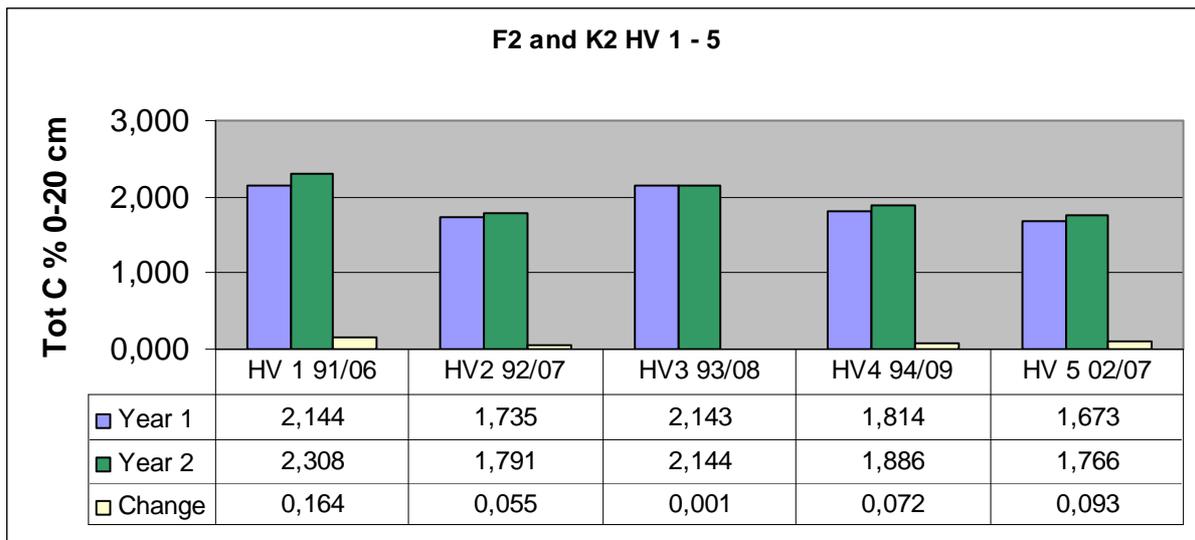


Figure 38. Total carbon content and changes in the top soils after three crop rotations with manure and one crop rotation in HV 5

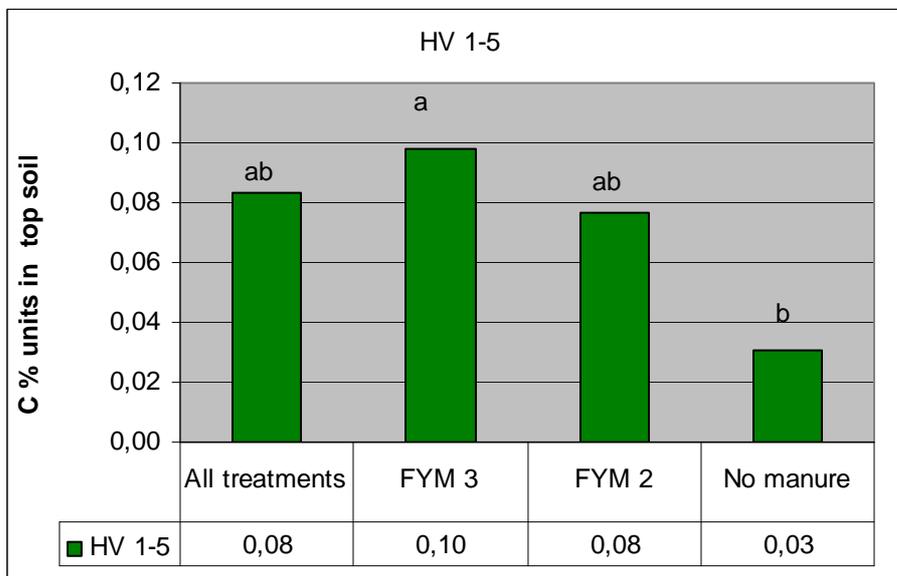


Figure 39. Changes in the amounts of total carbon in the top soil after three crop rotations, average of all treatments, with high manure (FYM 3), normal farm manure (FYM 2) and no manure.

Influence of composted and not composted manure

In HV 1 and HV 5 the treatments with composted manure, in comparison with the use of not composted manure, exhibited a stronger increase in the carbon content ($P < 0,1$) (**figure 40**).

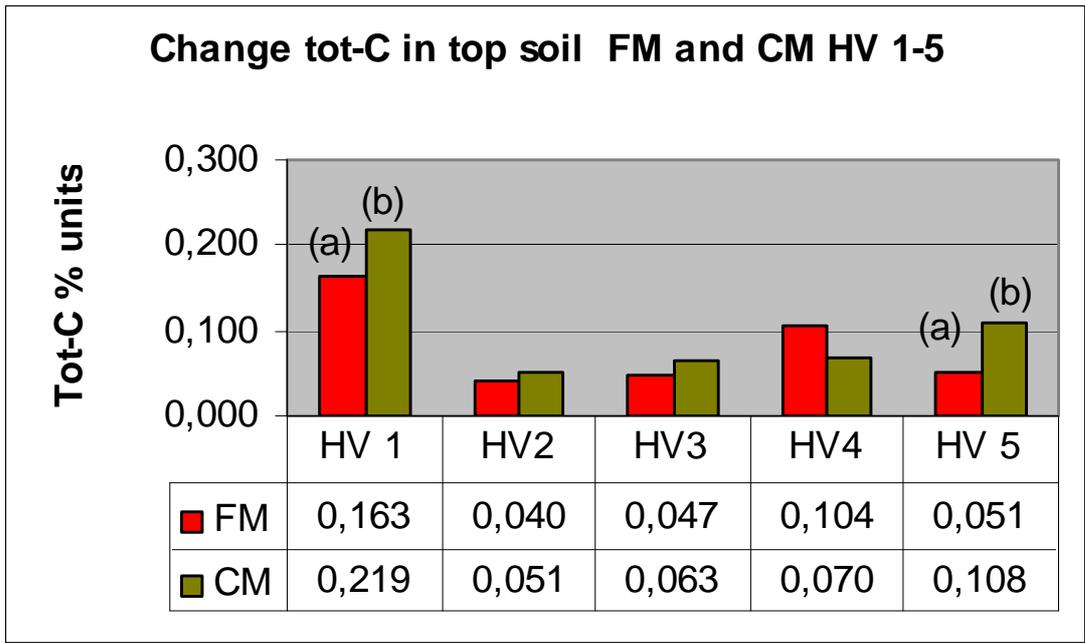


Figure 40. Change of total carbon in top soil from 1991 - 2005, averages for not composted (FM) and composted manure (CM)

In HV 1 the total carbon content in top soil was studied each year (**figure 41**). The carbon content increased steadily and with a higher increase in the soils treated with composted manure.

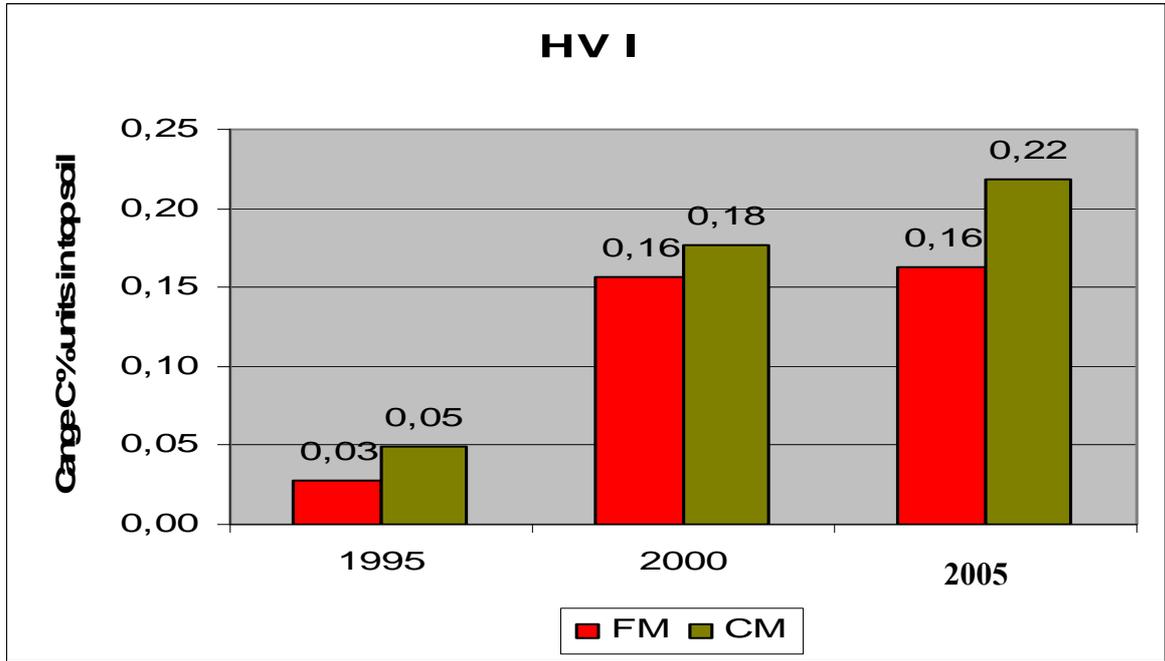


Figure 41. Change of total carbon in top soil from 1991 – 1995, 1991 – 2000 and 1991 – 2005, averages for not composted (FM) and composted manure (CM).

Influence of biodynamic preparation on total carbon in topsoil

In HV 4 and HV 5 the carbon content increased as average higher ($P < 0,1$) in the treatments with compost and biodynamic preparations compared with use of not composted manure (figure 42). In HV1, with composted manure level of 25 tons per ha (C II) the top soil carbon

increased during every crop rotation period and with significant higher level in the BDP treatments ($P < 0,05$) the years 1995 and 2005 (figure 43).

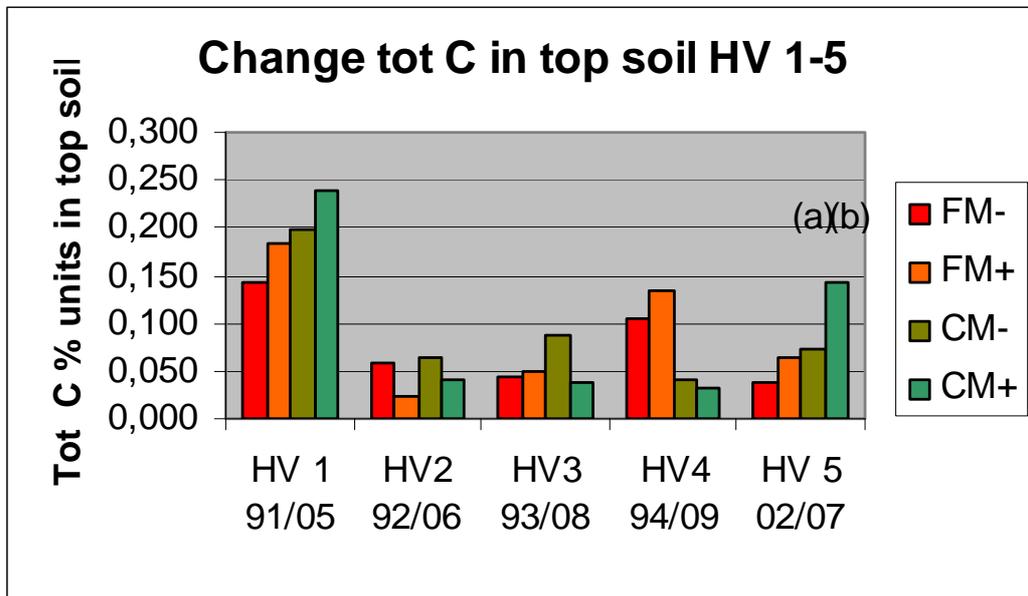


Figure 42. Change of total carbon in top soil in HV 1 from 1991 – 1995, 1992 – 2006, 1993-2008, 1994-2009 and HV 5 1992 – 2007 for not composted (FM) and composted manure (CM) without (-) and with (+) use of biodynamic preparation (BDP).

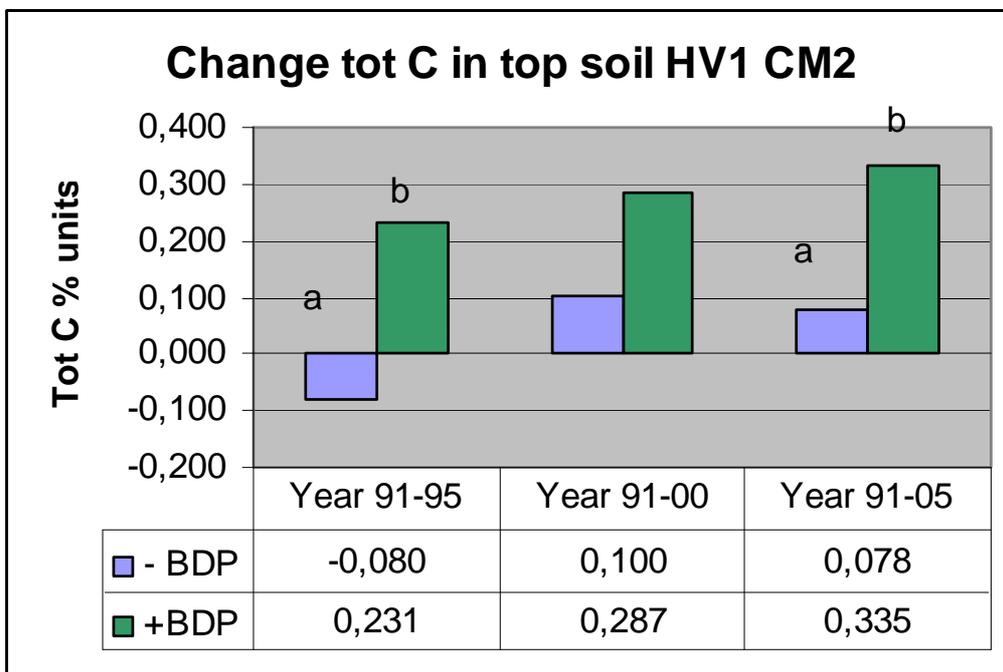


Figure 43. HV1 with composted manure level of 25 tons per ha (C2) without (-BDP) and with (+BDP) biodynamic preparations.

Carbon balance in a sustainable system with clover grass ley

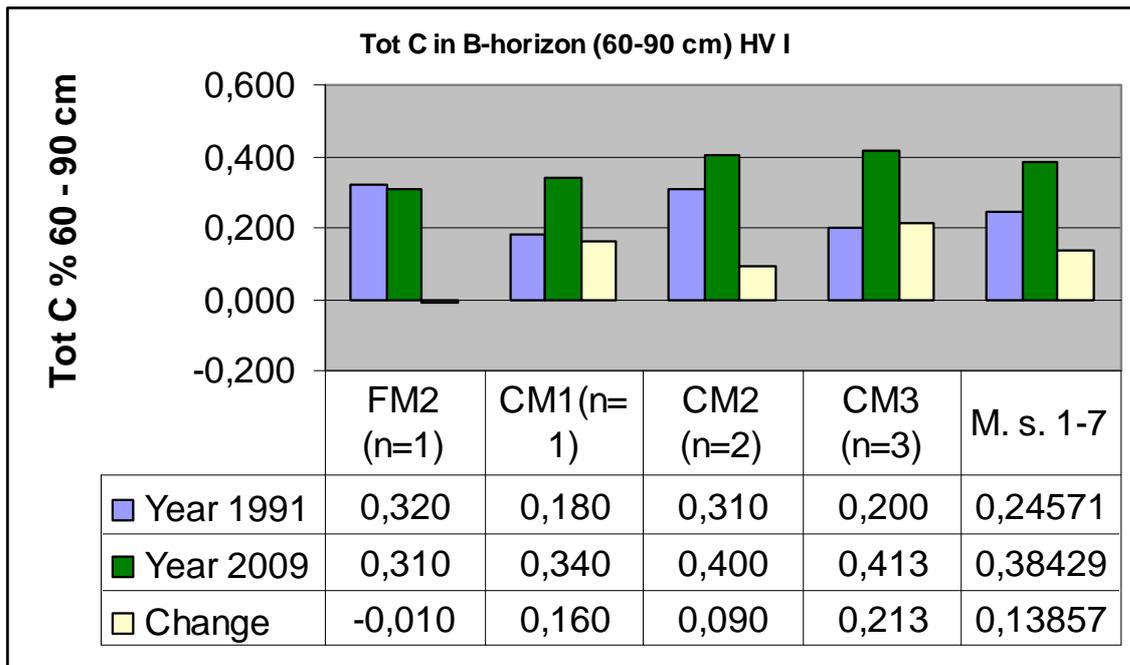


Figure 45. Measured total carbon in the deeper soil layer (60 – 90 cm), HV1 1991 and 2009.

Number of earthworms

Results from field trial HV 1



Figure 46. Counting the number of earthworms

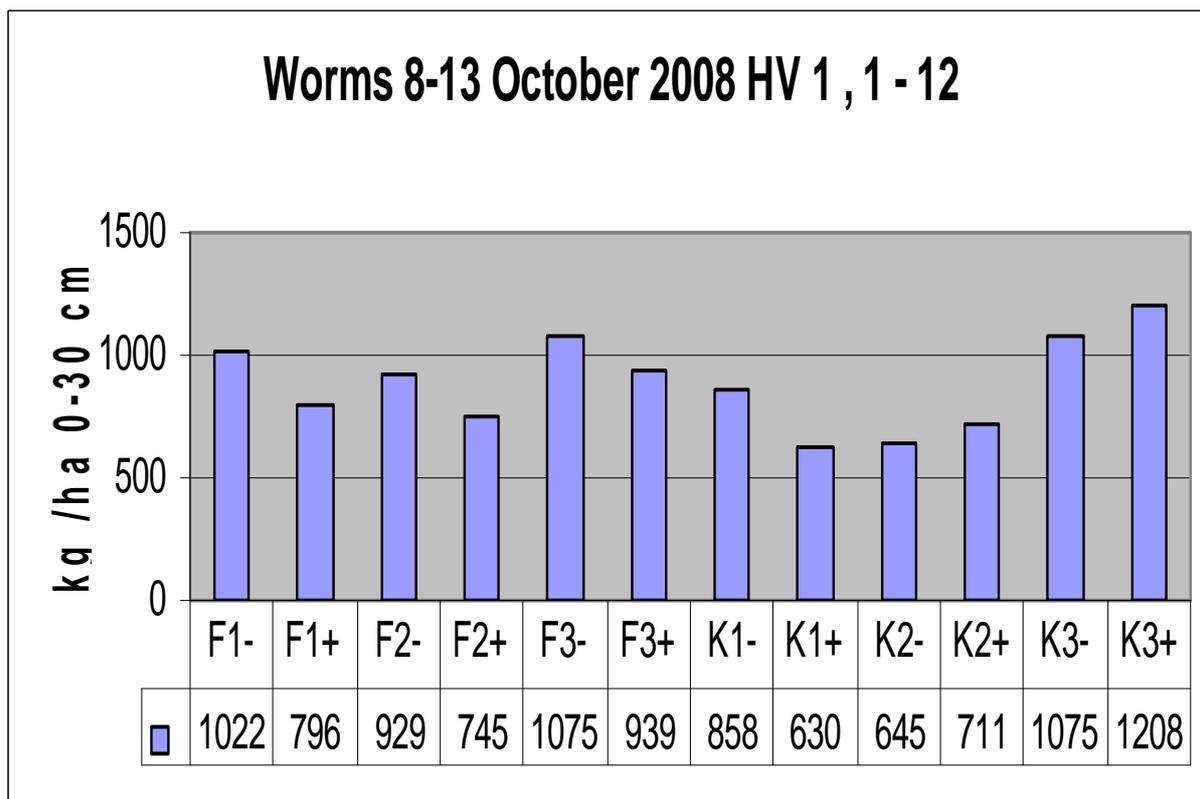


Figure 47. Total biomass of earth worms, kg/ha, in upper soil layer, 0-30 cm

In 2008 the biomass of earth worms in top soil was measured to be between 700 – 1200 kg per ha and increased with increasing amounts of manure. Treatment with the biodynamic preparations tended to lower the amount of earth worms when using fresh stable manure but not when using composted manure (figure 47). The worm activity measured 2006 as counted worm holes per m² were significantly higher with increasing amounts of manure and significant higher in the plots treated with biodynamic preparations (figure 48).

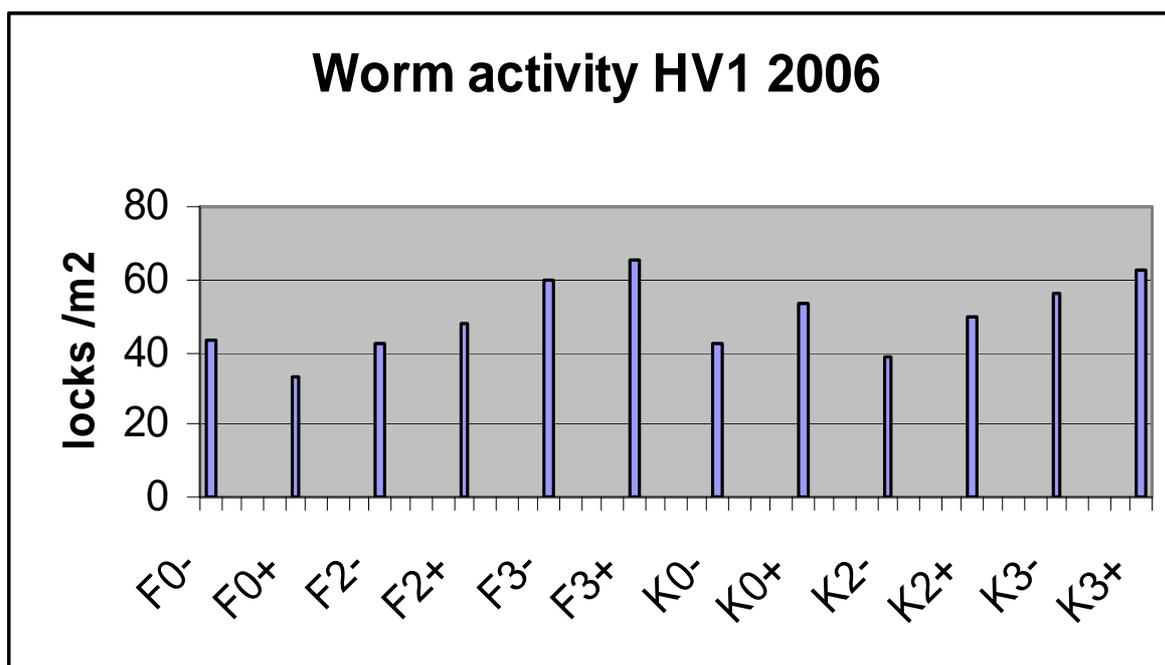


Figure 48. Number of holes of earthworms, holes/ m² in the upper soil, 0-30 cm

Wheat

Wheat, mainly as winter wheat, was grown during the whole study period 1991-2010 after clover grass.

Yields

The yield was evaluated for the different treatments in HV1, HV2, HV3, HV4 and HV5.

Influence of type of manure

Out of the total of 19 seasons the yield was higher during 14 seasons with the use of composted manure compared without composting, with an average increase for the whole period of 3, 5 % (**Figure 49**). In order to study the trends over time, the effects of yield after three years clover grass ley as in 1992, or with two years clover grass ley as a precrop need to be excluded



Figure 49. Yield of Winter wheat without (F) and with composting (K) of manure 1992 - 2010

Table 9. Yield of Winter wheat (85 % dm) without (F) and with composting (K) of manure 1992 -2010

		F	K
1992	HV1	5 253	5 307
1993	Hv2	3 003	2 716
1994	HV3	2 803	3 099
1995	HV4	2 204	2 095
1996	HV1	3 527	3 516
1997	HV2	3 050	3 285
1998	HV5	2 971	2 928
1999	HV3	2 774	2 938

2000	HV4	2 739	2 777
2001	HV1	2 980	3 015
2002	HV2	4 694	4 957
2003	HV5	3 949	4 096
2004	HV3	1 715	2 127
2005	HV4	4 211	4 385
2006	HV1	2 887	2 933
2007	HV2	2 984	2 737
2008	HV5	2 559	2 737
2009	HV3	2 455	2 963
2010	HV4	4 426	4 736

Influence of the biodynamic preparations

In plots treated with the biodynamic preparations the yields as an average were higher during 11 out of the 19 seasons, and of this the yield during 5 years was significantly higher (**Figure 50 and Table 10**). Three seasons the yields in the plots treated with the biodynamic preparations were lower and one year this decrease in yield was significant. The differences were higher during the first 6 years (average 5 %) compared to the whole period (average 2 %).

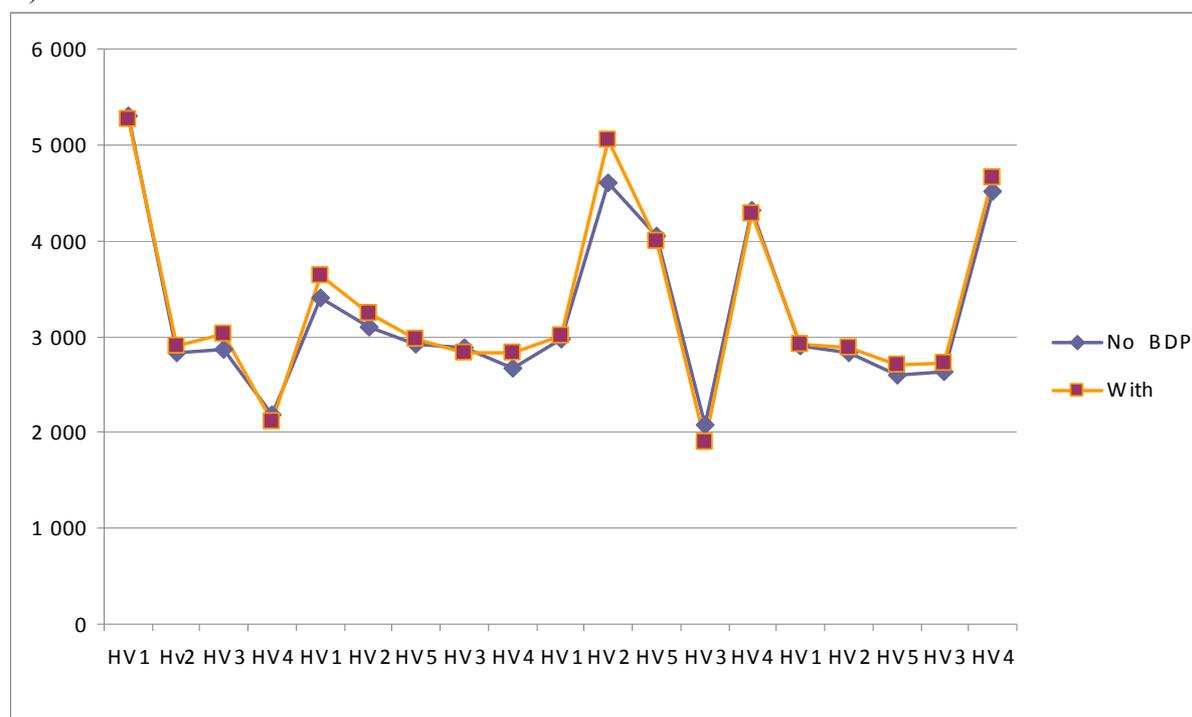


Figure 50. Yields, Winter wheat, in plots treated with compost and without (No BDP) or with the use of BD preparations (With) 1992 -2010

Table 10. Yields, Winter wheat, in plots treated with compost and without (No BDP) or with the use of BD preparations treatments (With BDP) 1992 -2010

		No BDP	With BDP
1992	HV1	5 293	5 261
1993	Hv2	2 822	2 898
1994	HV3	2 871	3 028
1995	HV4	2 189	2 110
1996	HV1	3 412	3 631
1997	HV2	3 099	3 237
1998	HV5	2 926	2 973

1999	HV3	2 880	2 832
2000	HV4	2 677	2 839
2001	HV1	2 980	3 015
2002	HV2	4 601	5 049
2003	HV5	4 042	4 003
2004	HV3	2 071	1 900
2005	HV4	4 309	4 287
2006	HV1	2 893	2 927
2007	HV2	2 829	2 892
2008	HV5	2 598	2 698
2009	HV3	2 638	2 718
2010	HV4	4 506	4 655
Average		3 244	3 313
Relative			1,0213

Influence of the amounts of manure

Figure 51 describes the yields of winter wheat during the period 1993 to 2010 for the three manure levels and figure 52 show the differences between the yield with no FYM (FYM1) to winter wheat, with 25 alternative 30 tons (FYM 2) and 50 tons (FYM 3) per ha.

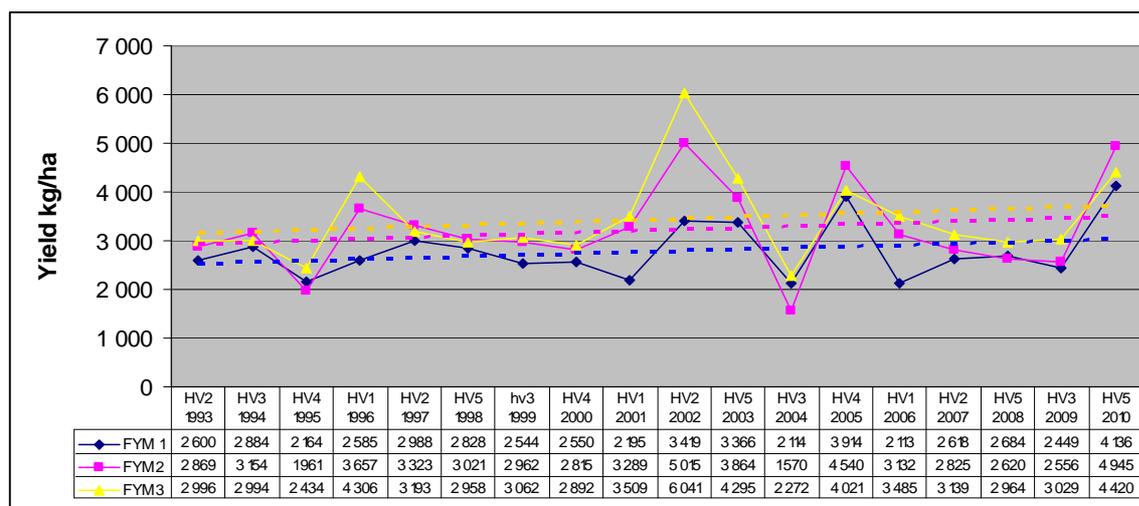


Figure 51. Yields of Winter wheat, in plots treated with low (FYM 1), normal (FYM 2) and high (FYM 3) level of manure (compost and without composting treatments) 1993 - 2010

The mean yield 3 445 kg per ha in FYM 3 is significantly higher than the not manured treatment FYM1 ($P < 0,05$) and the normal manure level in FYM 2 is slightly higher ($P < 0,1$) in comparison with the unmanured plots according to **figure 52**.

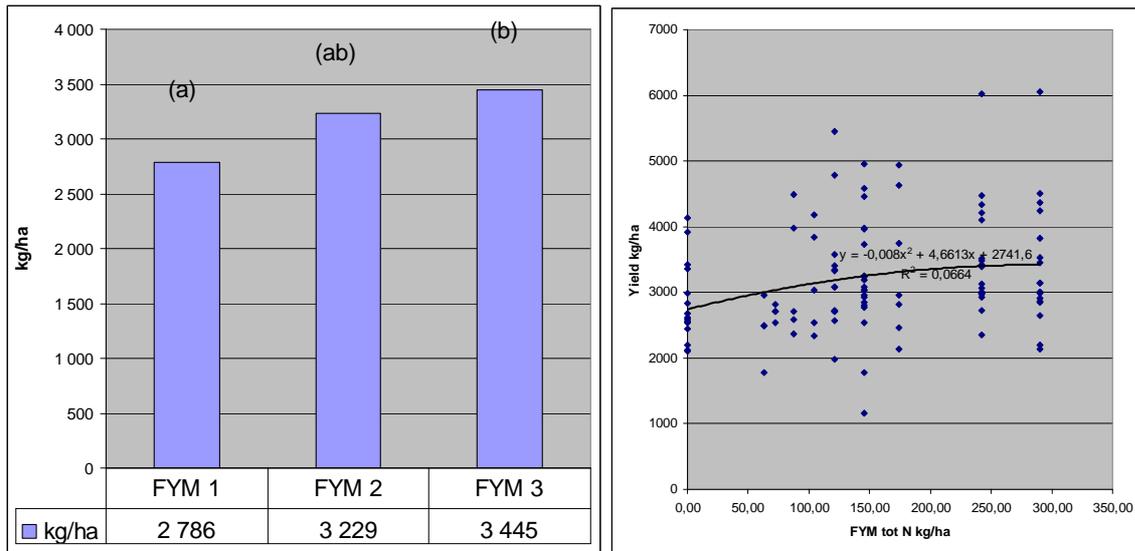


Figure 52. (Left) The average yields, winter wheat, in plots treated with low (FYM 1), normal (FYM 2) and high (FYM 3) level of manure (compost and without composting treatments) 1993 -2010. Figure 53. (Right) The relation between the total nitrogen content in applied manure and the yields of winter wheat.

The correlation between total nitrogen in applied manure (tot N kg/ha) and the yield of winter wheat (kg/ha) for the five experimental field 1993 - 2010 is presented in figure 53.

The figure 54 describes the over all yield in not composted and composted treatments 1993 to 2010 with a regression line indicating a very weak tendency for increased yield during the period 1993 -2010, ($y=26+2853$, $R^2=0,08$ and $y=42+2823$, $R^2=0,03$ respectively). The yields were higher in plots manured with composted manure in 1994, 1997, 2004, 2005, ($P<0,1$) and significantly higher in 2009, ($P<0,05$).

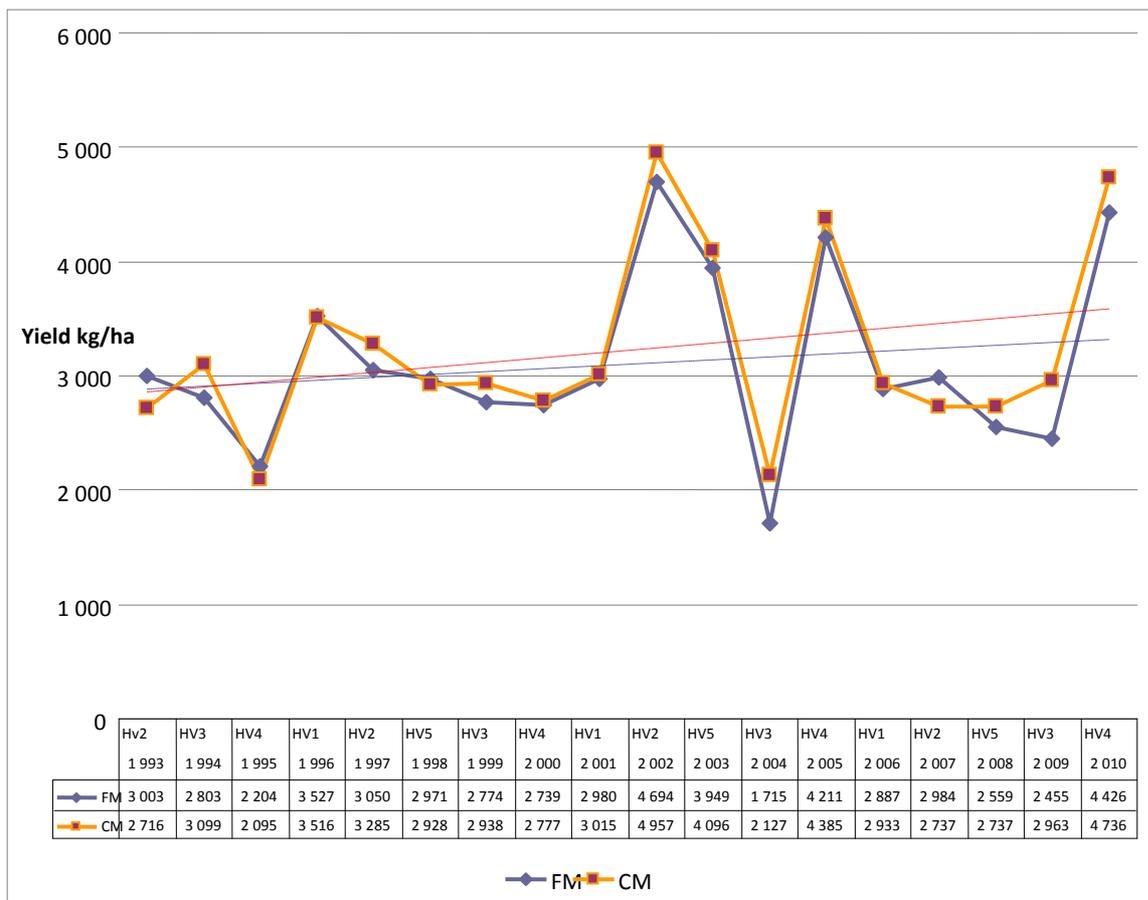


Figure 54. The yields of winter wheat during the period 1993 to 2010 in plots manured with not composted and composted manure. Dotted lines indicate the changes in yield levels.

The amount of 30 tons composted manure is comparable with 50 tonnes of fresh manure. The yield was as average higher in the normal manure level CM 2 (30 tons) in comparison with the fresh manure treatments FM3 (50 tons) in HV 4 and 5 (**figure 55**). Fresh manure, FM 2, (30 tons per ha) gave a higher yield than the low amount of manure in CM 1 (18 tons manure per ha) This is confirmed **figure 56** where CM 1 is compared to FM 2 and CM2 is compared to FM 3 for the six years with winter wheat 1998 to 2010.

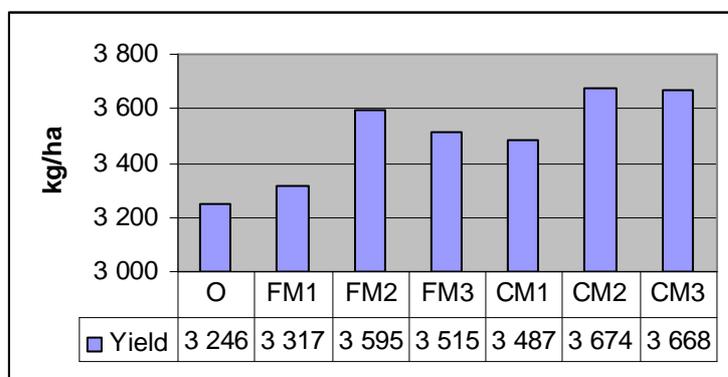


Figure 55. The average yield of winter wheat in not fertilized plots (0) and the three levels of fresh manure treatments (FM1, FM2 and FM3) and the three levels of composted manure in HV 4 and 5.

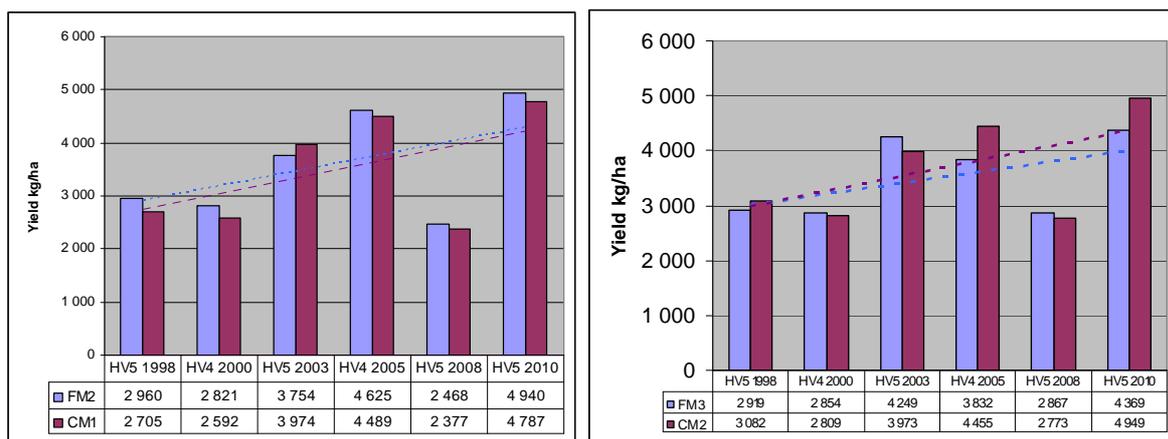


Figure 56. The wither wheat yield in not fertilized plots and the three levels of fresh manure treatments (FM1, FM2 and FM3) and the three levels of composted manure in HV 4 and 5.

Carrots

Carrots were grown during four seasons; 2004 in HV3, 2006 and 2007 in HV1 and 2008 in HV5. In 2004 two different varieties were compared.

The investigations during 2004 were more preliminary aiming to screen possible future studies. The trial field HV3 was heavily infested with cornflower, *Centaurea cyanus* and this influenced the development of the seeds. Carrots cultivated in soil fertilised with fresh stable manure were a little bigger and contained more sugar than carrots fertilised with compost. The rate of decomposition was higher in carrots fertilised with fresh stable manure where as the content of nitrate and free amino acids were higher in carrots fertilised with compost. The quality indices did not differ between fresh and composted manure. With increasing amounts of manure the indices decreased in carrots fertilised with fresh manure. The main reason was higher rates in the extract decomposition but lower amounts of sugars also contributed to decreasing indices. The choice of variety had a major effect on the carrots while treatment with the biodynamic preparations did not effect the properties.

During 2006, 2007 and 2008 only one variety was cultivated. The carrots were harvested three times per season (**Table 11**). The carrots grown during 2008 in HV5 suffered from drought and had great difficulties to germinate and to grow deeper than 10 cm into the soil. Due to poor germination not all plots had carrots to harvest during 2008 or some plots had very few at harvest 3. The carrots able to germinate were few but grew big if there were enough nutrients in the soil. Thus, carrots manured with easy soluble chicken manure grew faster and bigger in the beginning than carrots from the other treatments.

Table 11. Manuring regimes and date of treatments in carrot 2006, 2007 and 2008

Year	Manuring	Date of sowing	Harvest 1 Early	Harvest 2 Early-Normal	Harvest 3 Normal-Late
2006	According to plan+ Chicken manure to F1	9 th of May	26 th of August	10 th of September	24 th of September
2007	Chicken manure to F1	25 th of May	23 rd of August	23 rd of September	20 th of October
2008	According to plan+ Chicken manure to K1	1 st of June	18 th of August	15 th of September	4 th of October

Root size

The three parameters root weight, root length, root thickness and size of the collar represents the size related properties of the carrot root. The collar is the ring shaped scar after the stem on top of the root (**Figure 1**). The size of the collar indicates indirectly the size of the foliage and the width of the xylem (**Figure 1**).

Influence of harvest year and harvest periods

The season of 2007 differed from the other two seasons by lighter but longer carrot roots with smaller collars while the season of 2008 exhibited the thickest and shortest carrots (**Table 12**)

Table 12. Influence of harvest year on different root size parameters in carrots. Mean of all harvests during the season. Numbers followed by the same letter within a column are not significantly different from each other.

Year	N	Weight, g	Length, mm	Density, g/cm	Maximum thickness, mm	Mean thickness, mm	Collar size, mm
2006	144	68,2 a	108,1 b	6,0 b	35,6 a	26,6 b	16,1 a
2007	144	56,1 b	118,4 a	4,3 c	30,0 b	21,0 c	10,6 b
2008	77	69,1 a	76,6 c	8.2 a	36,6 a	30,6 a	16,4 a

In general all size related parameters increased significantly during the harvest season expressing the smallest values at harvest number 1 and the largest at harvest number 3 (**Data not shown**).

Influence of type of manure

The differences in root size between the manured plots were small and mainly caused by the situation during 2008 described earlier. Unmanured carrots were lighter and had smaller collars but were of equal length as manured carrots (**Table 13**). Carrots grown in soils manured with composted manure had smaller collars in comparison with samples manured with pelleted chicken manure (**Table 13**).

Table 13. Influence of type of manure on different root size parameters in carrots. Mean of all harvests and seasons. Numbers followed by the same letter within a column are not significantly different from each other.

Treatment	N	Weight, g	Length, mm	Collar size, mm
No manure	58	49,7 b	103,4 a	12,8 c
Chicken manure	63	73,0 a	104,8 a	14,8 a
Fresh manure	61	66,4 a	107,3 a	14,2 ab
Composted manure	60	66,1 a	106,5 a	13,7 b

The effect of the harvest season 2008 is most obvious at harvest number 1 (**Figure 27**). At harvest number 2 carrots manured with fresh stable manure had increased in size and at harvest 3 (**Figure 27**) the differences between the treatments are more in correlation with the seasons of 2006 and 2007 (**Figure 27**). During the season of 2007 no stable manure was used. Despite of this, carrots grown in soil previously manured with composted manure could match carrots fertilised with chicken manure. In soil previously manured with fresh stable manure the final size of the carrots was not bigger than the carrots grown in unmanured soil (**Figure 27**)

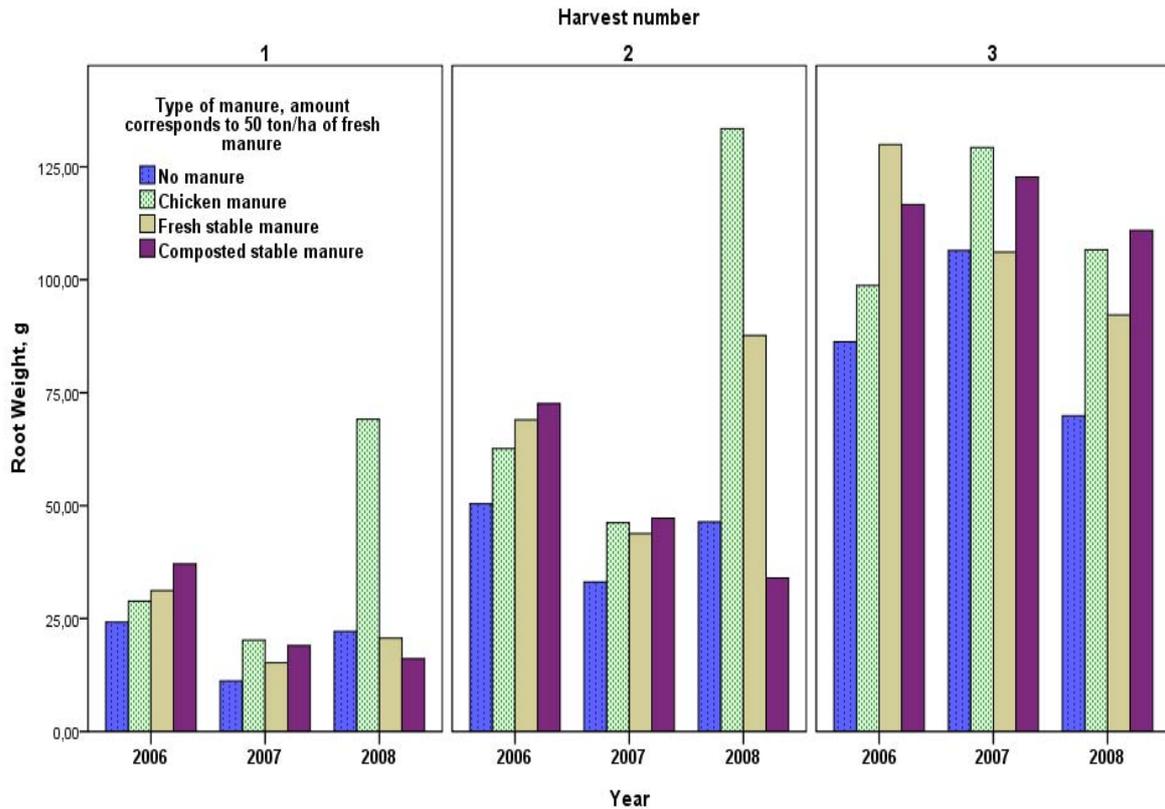


Figure 57. Influence of type of manure on root weight at the three harvest periods during 2006, 2007 and 2008.

If excluding the year 2008 from the results, the growth of the carrots developed according to table 14. Unmanured carrots were generally smaller than manured, with the exception of root length and collar size at harvest 3 (**Table 14**) At harvest 1 size related parameters were significantly higher in carrots manured with compost in comparison with unmanured carrots (**Table 14**). At harvest number 1 carrots manured with compost also exhibited longer carrots in comparison with carrots manured with fresh stable manure or with pelleted chicken manure (**Table 14**). At harvest number 2 carrots manured with fresh manure had reached a size equal to that of carrots manured with compost whereas carrots manured with chicken manure still was significantly shorter (**Table 14**). At harvest number 3 the sizes of the manured carrots were the same regardless of type of manure with the exception that carrots manured with chicken manure still was significantly shorter (**Table 14**).

Table 14. Influence of type of manure on different root size parameters in carrots. Mean of harvests during the seasons of 2006 and 2007. Values followed by the same letter within the same harvest number are not significantly different from each other.

Harvest number	Treatment	N	Weight, g	Length, mm	Density, g/cm	Collar size, mm
1	No manure	16	17,7 c	96,3 b	1,9 b	10,5 b
	Chicken manure	16	24,6 ab	97,5 b	2,5 a	11,6 a
	Fresh manure	16	23,2 b	99,1 b	2,4 a	11,4 ab
	Composted manure	16	28,1 a	105,0 a	2,7 a	11,9 a
2	No manure	16	41,8 b	104,6 b	4,0 b	11,6 b
	Chicken manure	16	54,4 a	105,5 b	5,2 a	12,8 ab
	Fresh manure	16	56,4 a	111,3 a	5,1 a	13,4 a
	Composted manure	16	59,9 a	112,8 a	5,4 a	12,7 ab
3	No manure	16	96,4 b	132,0 ab	7,2 b	15,4 a
	Chicken manure	16	114,0 a	126,6 b	8,9 a	16,2 a
	Fresh manure	16	118,0 a	135,3 a	8,7 a	16,3 a
	Composted manure	16	119,7 a	130,5 ab	9,1 a	15,8 a

Influence of amount of manure

The unmanured plots had significantly lower weight and collar size than the corresponding manured plots (**Table 15**). There was however no differences in root length between the treatments when comparing all samples (**Table 15**).

Table 15. Influence of type of manure on different root size parameters in carrots. Mean of all harvests and seasons. Numbers followed by the same letter within a column are not significantly different from each other.

Amount of manure	N	Weight, g	Length, mm	Collar size, mm
No manure	58	49,7 b	103,4 a	12,8 b
25 tons/ha fresh or composted manure	123	62,7 a	105,5 a	14,0 a
50 tons/ha fresh or composted manure	121	66,3 a	106,9 a	14,2 a

Excluding the season of 2008 and expressing the different harvests gives a more detailed picture (**Table 16**). Plots manured with 50 tons/ha exhibited heavier carrots than plots manured with 25 tons/ha, harvest 2 excluded (**Table 16**). The density of the roots was higher at all harvests in carrots manured with 50 tons/ha (**Table 16**). The length of the carrots was significantly higher in plots manured with 50 tons/ha at the first two harvests (**Table 16**).

Table 16. Influence of amount of manure on different root size parameters in carrots. Mean of harvests during the seasons of 2006 and 2007. Values followed by the same letter within the same harvest number are not significantly different from each other.

Harvest number	Treatment	N	Weight, g	Length, mm	Density, g/cm	Collar size, mm
1	No manure	16	17,7 c	96,3 b	1,9 b	10,5 b
	25 tons/ha manure	32	21,6 b	97,5 b	2,2 b	11,6 a
	50 tons/ha manure	32	25,6 a	102,0 a	2,5 a	11,6 a
2	No manure	16	41,8 b	104,5 b	4,0 c	11,6 b
	25 tons/ha manure	32	53,1 a	111,1 b	4,8 b	12,6 ab
	50 tons/ha manure	32	58,2 a	112,0 a	5,2 a	13,1 a
3	No manure	16	96,4 c	132,0 a	7,2 c	15,4 a
	25 tons/ha manure	32	107,3 b	132,6 a	8,1 b	16,3 a
	50 tons/ha manure	32	118,9 a	132,9 a	8,9 a	16,1 a

Influence of biodynamic preparations

Significant differences in root weight between plots treated or not treated with biodynamic preparations were found at harvest number 2 during 2006 and harvest 3 during 2007 (**Figure 57**). In both cases the untreated samples exhibited heavier roots. During the season of 2008 no general differences were found (**Figure 57**).

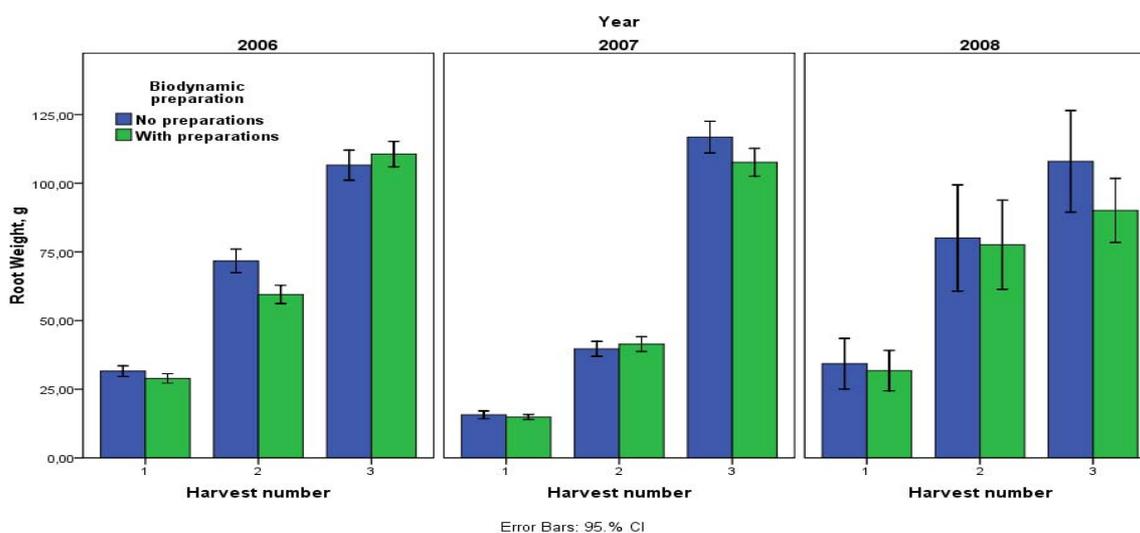


Figure 58. Effect of biodynamic preparation on carrot weight. Mean of all treatments.

When comparing manuring systems during the season 2006 and 2007 most significant differences occurred in soils fertilised with composted manure although differences was recorded also among unmanured samples and among samples fertilised with fresh stable manure (**Figure 58**).

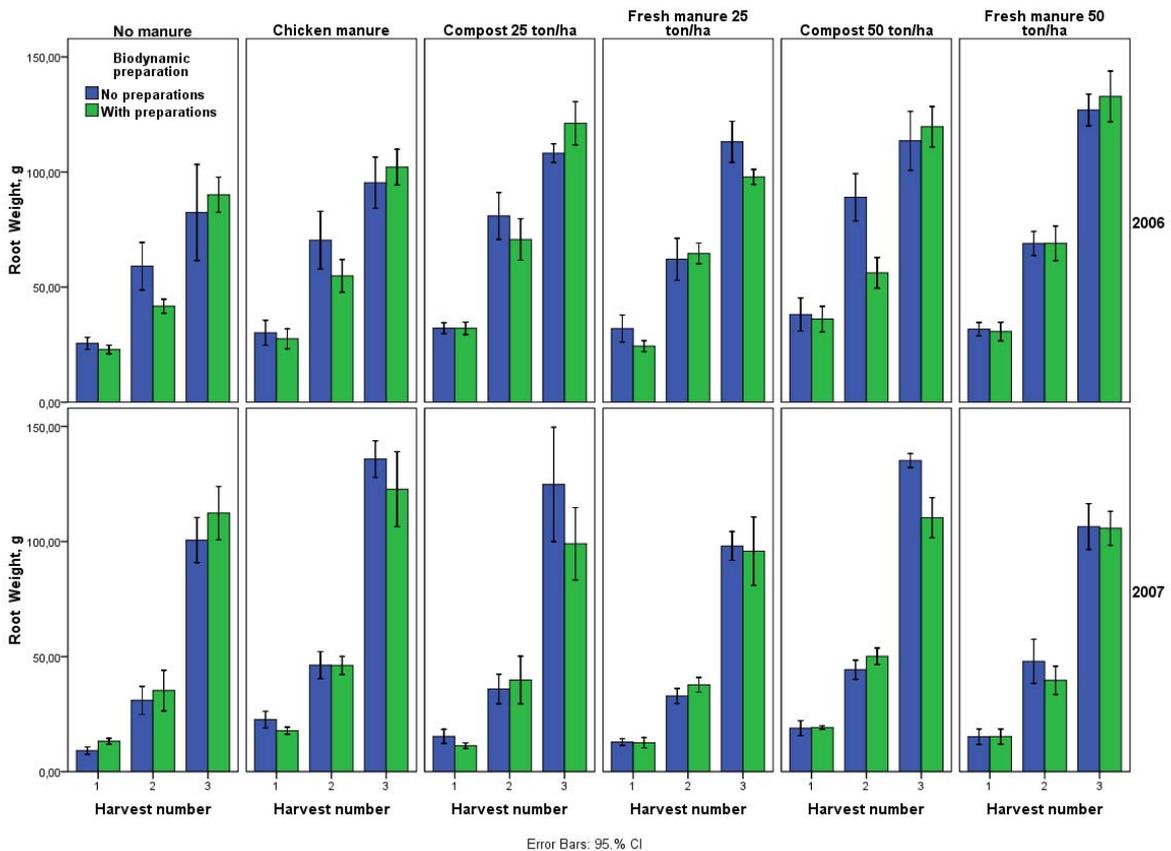


Figure 59. Effect of biodynamic preparation on carrot weight. Mean of samples from 2006 and 2007.

Root shape

Root cylindricity and root bluntness is used to describe the morphological properties of the carrot root. A value closer to 1 represents a more cylindrical shape while a value closer to 0 represents a more conical shape (**Figure 4**). The shape of the root tip is determined by the value of root bluntness. A value closer to 1 represents a more rounded tip while a value closer to 0 represents a more pointed tip, (**Figure 3**).

Influence of harvest year and harvest period

Root cylindricity was significantly higher during 2008 in comparison with 2006 and 2007 (**Figure 59**). During 2007 root cylindricity increased during the harvest season while it decreased at the end of the seasons in 2006 and 2008 (**Figure 59**).

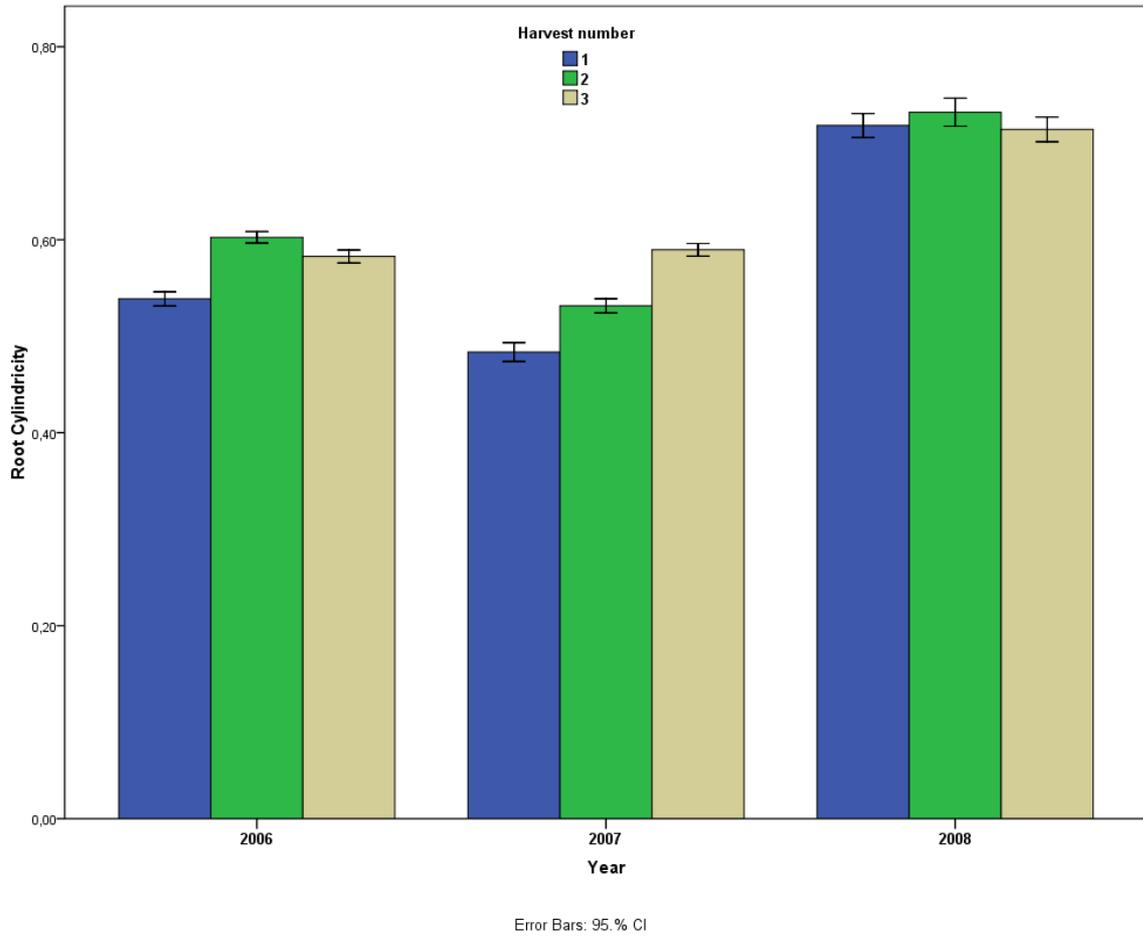


Figure 60. Effect of harvest period on root cylindricity. Mean of all samples 2006, 2007 and 2008.

Root bluntness exhibited an opposite development during 2007 in comparison with root cylindricity as it decrease during the harvest season (**Figure 60**). The extraordinary high values for root bluntness in 2008 were due to the special soil conditions mentioned earlier. The decreasing values concerning root bluntness during 2006 was probably due to a stronger increase in root length during the second half of this season.

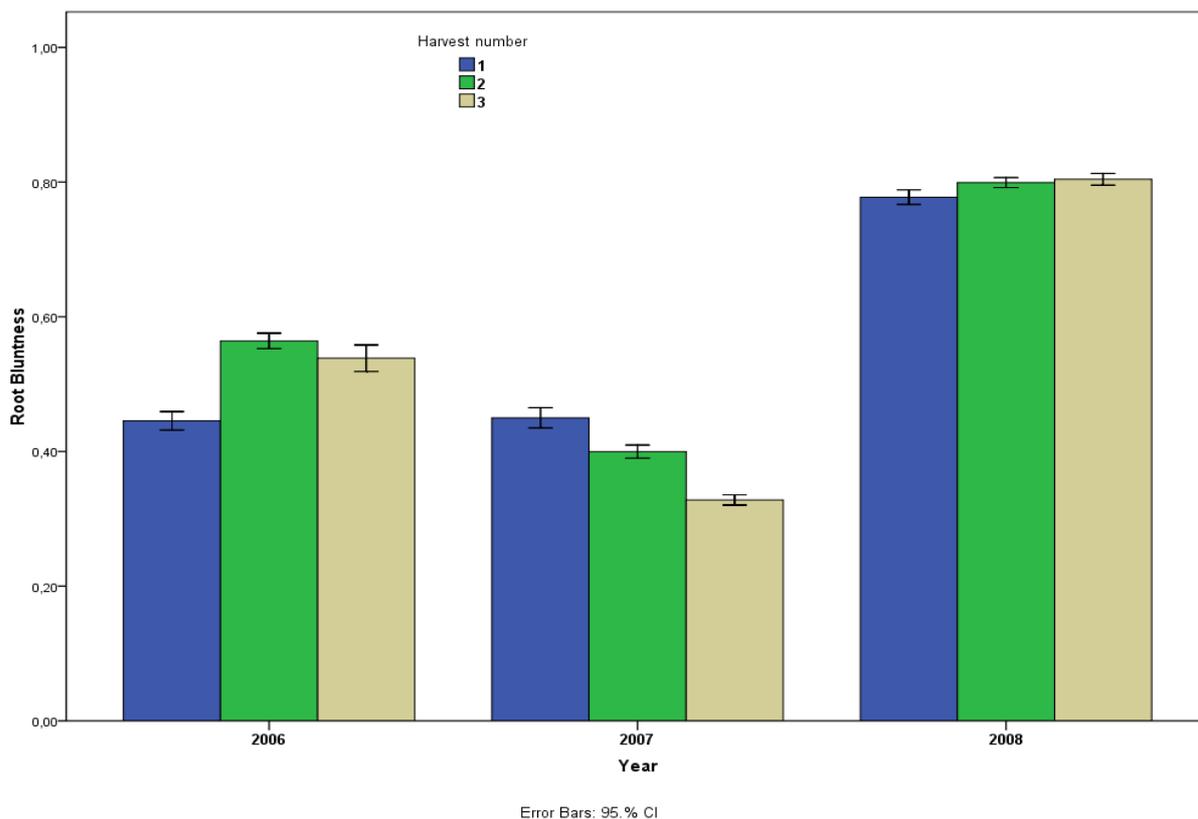


Figure 61. Effect of harvest period on root bluntness. Mean of all samples 2006, 2007 and 2008.

Influence of type of manure

If the carrots were grown in soils manured with chicken manure root bluntness was high already at harvest 1 (**Table 15**). Root cylindricity was low in carrots cultivated in unmanured soils, regardless of harvest period (**Table 15**). Carrots grown in soil manured with compost had high root cylindricity, especially at harvest 1 and harvest 3 (**Table 15**).

Table 17. Influence of type of manure on different morphological parameters in carrots. Mean of harvests during the seasons of 2006 and 2007. Values followed by the same letter within the same harvest number are not significantly different from each other.

Harvest number	Treatment	N	Root Bluntness	Root Cylindricity
1	No manure	16	0,45 ab	0,49 c
	Chicken manure	16	0,48 a	0,52 ab
	Fresh manure	16	0,43 b	0,49 bc
	Composted manure	16	0,46 ab	0,54 a
2	No manure	16	0,48 a	0,55 b
	Chicken manure	16	0,50 a	0,58 a
	Fresh manure	16	0,48 a	0,57 ab
	Composted manure	16	0,48 a	0,57 ab
3	No manure	16	0,45 a	0,58 b
	Chicken manure	16	0,42 a	0,59 b
	Fresh manure	16	0,42 a	0,58 b
	Composted manure	16	0,42 a	0,61 a

Influence of amount of manure

Carrots grown in unmanured soils had lower root cylindricity at harvest 1 and harvest 3 in comparison with carrots grown in soil manured with 50 tonnes/ha of fresh or composted stable manure (**Table 16**). At harvest 3 root cylindricity was significantly higher in carrots manured with 50 tons/ha in comparison with carrots manured with 25 tons/ha (**Table 16**). The amount of manure did not influence root bluntness (**Table 16**).

Table 18. Influence of amount of manure on root shape in carrots. Mean of harvests during the seasons of 2006 and 2007. Values in a column followed by the same letter within the same harvest number are not significantly different from each other.

Harvest number	Treatment	N	Root Bluntness	Root Cylindricity
1	No manure	16	0,45 a	0,49 b
	25 tons/ha manure	32	0,43 a	0,52 a
	50 tons/ha manure	32	0,45 a	0,51 a
2	No manure	16	0,48 a	0,55 a
	25 tons/ha manure	32	0,47 a	0,56 a
	50 tons/ha manure	32	0,48 a	0,57 a
3	No manure	16	0,45 a	0,58 b
	25 tons/ha manure	32	0,44 a	0,58 b
	50 tons/ha manure	32	0,42 a	0,60 a

Influence of biodynamic preparations

Treatment with biodynamic preparations significantly decreased root bluntness at harvest 2 and harvest 3 during 2007 and harvest 1 during 2008 (Data not shown). The same treatment significantly increased root cylindricity at harvest 2 in 2007 and 2008 (data not shown) but significantly decreased root cylindricity at harvest 3 (**Figure 38**).

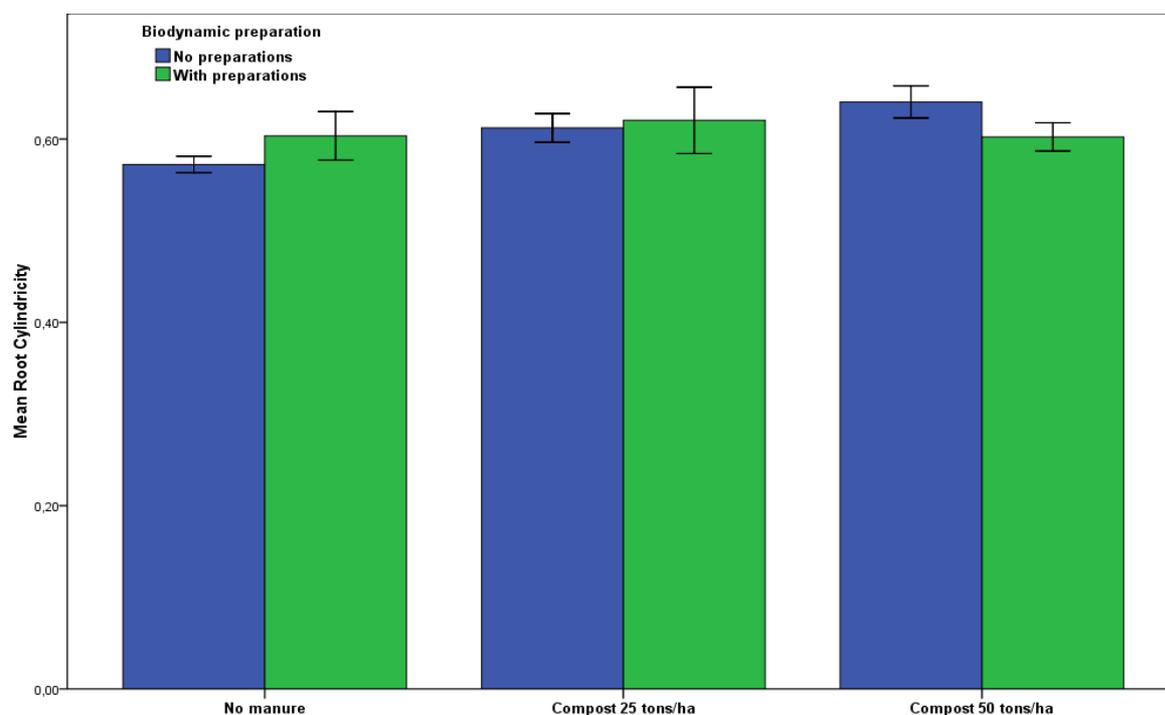


Figure 62. Influence of biodynamic preparations and amount of compost systems on root cylindricity. Mean of samples from harvest 3 in 2006, 2007 and 2008.

Sugars

The carrot root mainly store sugar as fructose, glucose and sucrose.

Influence of harvest year and harvest period

The year of 2007 exhibit a typical development in the amounts of sugars during the harvest season. During the year of 2006 the monosaccharide's do not decrease and sucrose does not increase as normal during the end of the harvest season indicating that the carrots do not develop into winter maturity. Also during the season of 2008 the amounts of fructose and glucose do not decrease in a typical way (**Figure 39**).

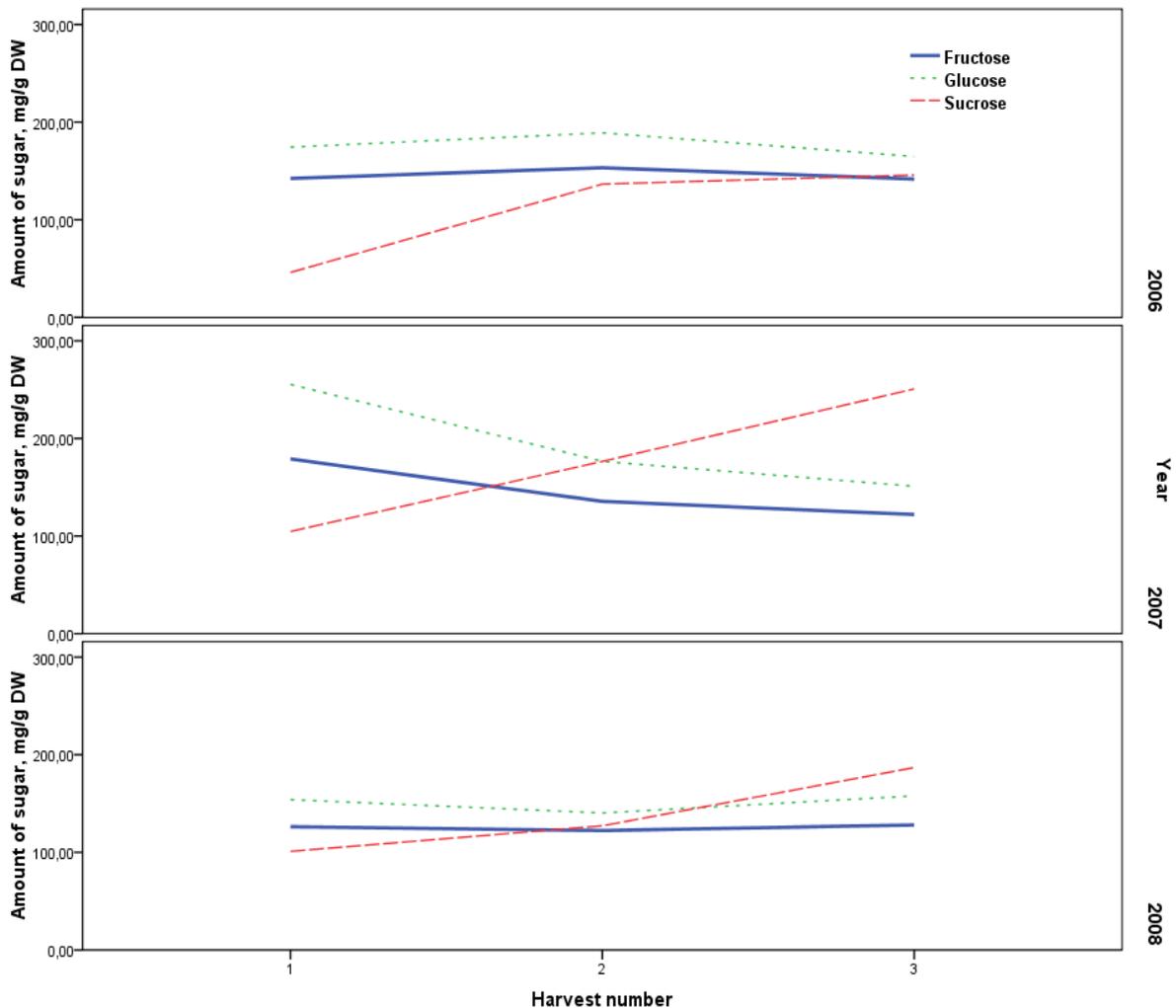


Figure 63. Development of the amounts of fructose, glucose and sucrose, mg/g DW, depending on harvest period and harvest year. Mean of all samples.

Influence of type of manure

The type of manure did not influence the amount of monosaccharides but at harvest 1 the amount of sucrose was significantly lower in samples grown in unmanured soil or in soil manured with fresh stable manure compared with carrots grown in soil manured with pelleted chicken manure (**Table 17**).

Table 19. Influence of type of manure on the amounts of sugars, mg/g DW, in carrots. Mean of harvests during all three seasons. Values followed by the same letter within the same harvest number are not significantly different from each other.

Harvest number	Treatment	N	Fructose	Glucose	Sucrose	Total sugar
1	No manure	20	155,2 a	211,9 a	65,9 c	433,0 a
	Chicken manure	20	153,3 a	196,8 a	102,3 a	452,9 a
	Fresh manure	20	162,5 a	210,7 a	82,5 bc	455,7 a
	Composted manure	20	156,9 a	204,0 a	91,1 ab	451,9 a
2	No manure	20	148,8 a	186,8 a	151,8 a	487,4 a
	Chicken manure	20	127,0 b	156,5 b	165,3 a	448,7 b
	Fresh manure	20	145,4 a	177,8 a	157,5 a	480,8 ab
	Composted manure	20	141,6 a	176,2 a	150,4 a	468,1 ab
3	No manure	20	134,1 a	162,1 a	190,3 a	486,5 ab
	Chicken manure	20	119,7 b	132,7 b	177,9 a	430,2 b
	Fresh manure	20	127,3 ab	151,4 a	196,6 a	475,3 ab
	Composted manure	20	134,2 a	169,9 a	207,3 a	511,4 a

Influence of amount of manure

The amount of manure significantly influenced the content of sugars during the first half of the harvest season. Unmanured carrots had significantly lower amounts of sucrose at harvest 1 (**Table 18**). Carrots manured with 25 tons of manure exhibited significantly low amounts of all sugars, especially at harvest 2 in comparison with carrots manured with 50 tons/ha (**Table 18**).

Table 20. Influence of amount of manure on the amounts of sugars, mg/g DW, in carrots. Mean of harvests during all three seasons. Values followed by the same letter within the same harvest number are not significantly different from each other.

Harvest number	Treatment	N	Fructose	Glucose	Sucrose	Total sugar
1	No manure	20	155,2 ab	211,9 a	65,9 b	433,0 ab
	25 tons/ha manure	40	145,8 b	193,5 a	70,1 b	409,5 b
	50 tons/ha manure	40	159,8 a	207,4 a	86,7 a	453,8 a
2	No manure	20	148,8 a	186,8 a	151,8 a	487,4 a
	25 tons/ha manure	40	136,8 b	170,2 b	135,5 b	442,5 b
	50 tons/ha manure	40	146,7 ab	177,0 ab	154,2 a	474,9 a
3	No manure	20	134,1 a	162,1 a	190,3 a	486,5 a
	25 tons/ha manure	40	136,6 a	166,8 a	199,8 a	503,1 a
	50 tons/ha manure	40	130,6 a	160,2 a	201,6 a	492,4 a

Influence of biodynamic preparations

The biodynamic preparation occasionally had a significant impact on the amounts of sugar in the carrots (**Figure 35**). In soils manured with fresh stable manure the preparations significantly increased the amounts of sucrose during the second half of the harvest season (**Figure 34**).

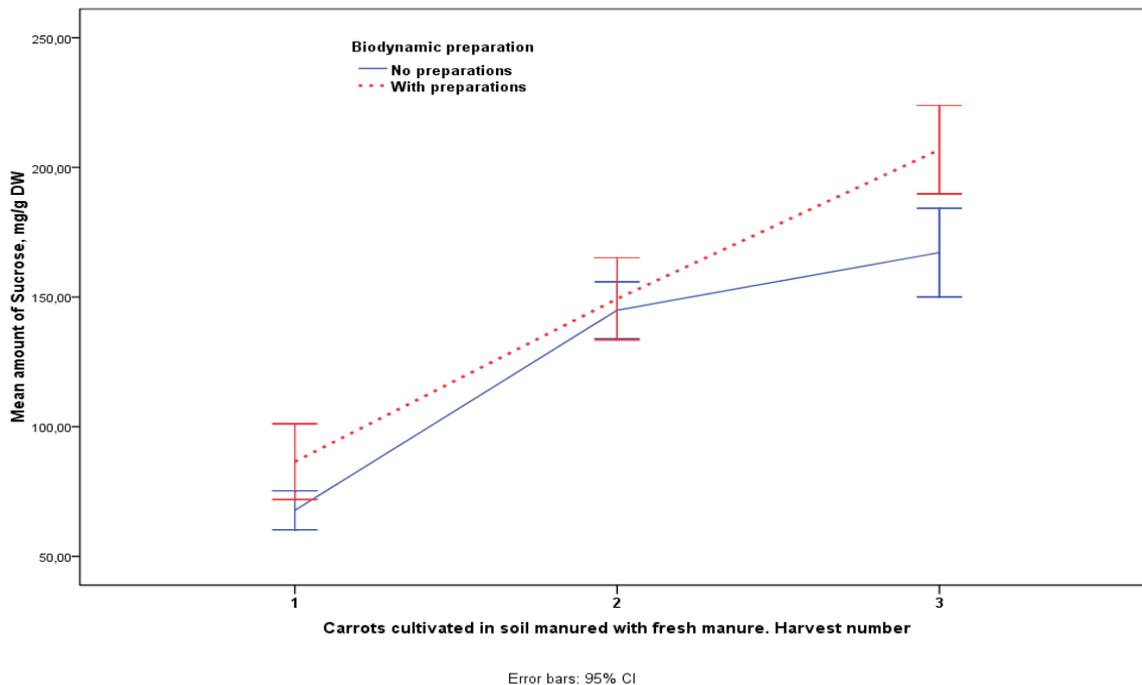


Figure 64. Changes in the amount of sucrose, mg/g DW, in carrots cultivated in soils manured with fresh stable manure, depending on harvest period and treatment with the biodynamic preparation

The effect of the biodynamic preparation, especially in the amounts of sucrose, interacted both with the fertilising system and with the annular situation (Figure 35).

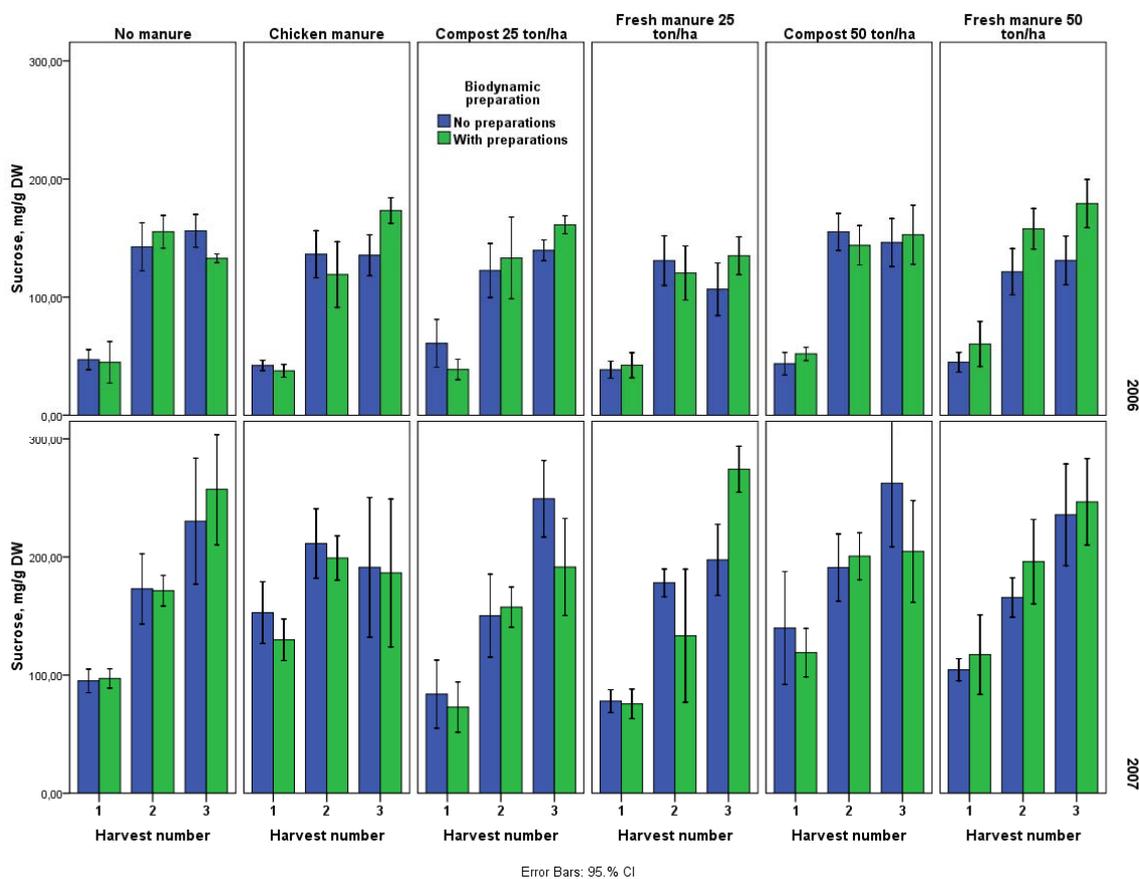


Figure 65. Influence of biodynamic preparations and fertilisation system on the amounts of sucrose, mg/g DW. Mean of samples from 2006 and 2007.

Bitter tasting agents

The variations in the amounts of polyacetylenes falcarindiol, falcarindiol-3-acetate and falcarinol exhibited only minor differences depending on cultivations measures whereas the seasonal parameters had a more obvious effect.

Influence of harvest year and harvest period

The amounts of polyacetylenes varied significantly between harvest seasons (Table 19). The season of 2006 exhibited low amounts of all polyacetylenes (Table 19). The year of 2007 had significantly lower amounts of falcarinol and 2008 significantly lower amounts of falcarindiol-3-acetate (Table 19)

Table 21. Influence of harvest year on the amounts of polyacetylenes, µg/g DW. Mean of all samples.

Year	Falcarindiol	Falcarindiol-3-acetate	Falcarinol	Total polyacetylenes
2006	450.6 b	46.5 a	100.0 b	611.6 b
2007	457.5 B	49.9 a	74.7 c	582.1 b
2008	554.4 a	26.8 c	118.2 a	746.0 a

High amounts of polyacetylenes were found most often at harvest number 2 (Table 20). The amounts of falcarindiol and total polyacetylenes were significantly higher at harvest number 2, the amounts of falcarindiol-3-acetate significantly lower at harvest 3 and the amounts of falcarinol significantly lower at harvest number 1 (Table 20)

Table 22. Influence of harvest period on the amounts of polyacetylenes, µg/g DW. Mean of all samples.

Harvest number	Falcarindiol	Falcarindiol-3-acetate	Falcarinol	Total polyacetylenes
1	458.1 b	49.7 a	69.0 c	602.5 b
2	573.1 a	52.6 a	115.5 a	759.8 a
3	396.3 b	30.4 b	94.81 b	521.6 c

Influence of type of manure

The type of manure influenced the amounts of all polyacetylenes with the exception of falcarindiol-3-acetate (Table 21). Carrots grown in soil manured with pelleted chicken manure had significantly lower amounts of falcarindiol, falcarinol and total polyacetylenes in comparison with samples from unmanured soils (Table 21). Pelleted chicken manure significantly lowered the amounts of falcarinol also in comparison with samples cultivated in soils manured with fresh or composted manure (Table 21).

Table 23. Influence of type of manure on the amounts of polyacetylenes, $\mu\text{g/g}$ DW. Mean of all samples.

Type of manure	Falcarindiol	Falcarindiol-3-acetate	Falcarinol	Total polyacetylenes
No manure	473.2 a	48.6 a	94.0 a	625.4 a
Chicken manure	411.7 b	43.3 a	70.7 b	540.9 b
Fresh stable manure	456.1 ab	49.3 a	88.4 a	603.1 ab
Composted stable manure	447.2 ab	47.8 a	87.3 a	582.3 ab

Influence of amount of manure

The applied amount of fresh stable manure or composted manure did not effect the amounts of polyacetylenes (**Table 22**)

Table 24. Influence of amount of manure on the amounts of polyacetylenes, $\mu\text{g/g}$ DW. Mean of all samples.

Amount of Manure	Falcarindiol	Falcarindiol-3-acetate	Falcarinol	Total polyacetylenes
No manure	460.8 a	42.5 a	90.8 a	612.6 a
25 ton/ha	497.8 a	46.3 a	100.3 a	648.4 a
50 ton/ha	477.8 a	43.2 a	93.9a	624.4 a

Influence of biodynamic preparations

The biodynamic preparations only occasionally influenced the amounts of polyacetylenes (**Table 23**). The only thing arguing for that the influence of the preparation is not a random effect is the fact that all significant effects occurred during harvest number 2.

Table 25. Influence of biodynamic preparations on the amounts of polyacetylenes, µg/g DW. Mean of all samples. Significant differences at $p < 0.05$ is indicated with **

Treatment	Harvest number	Biodynamic preparation	Falcarindiol	Falcarindiol-3-acetate	Falcarinol	Total polyacetylenes
No manure	1	No preparations	471.4	43.3	64.8	579.5
		With preparations	437.5	46.7	71.9	636.0
	2	No preparations	603.2	54.5	140.4	830.2
		With preparations	563.0	47.7	127.8	762.5
	3	No preparations	301.4	28.5	65.6	395.5
		With preparations	342.8	32.1	64.5	439.4
Chicken manure	1	No preparations	460.9	57.1	63.8	672.9
		With preparations	376.3	42.8	53.6	507.3
	2	No preparations	465.0	47.6	65.1	627.1
		With preparations	494.6	40.2	93.1**	642.7
	3	No preparations	440.7	36.5	114.6	591.8
		With preparations	391.2	33.3	78.8	503.3
Compost 25 ton/ha	1	No preparations	462.9	54.0	81.9	608.7
		With preparations	529.5	58.5	83.9	671.9
	2	No preparations	675.5	56.8	131.7	906.6
		With preparations	508.7**	48.1	113.8	675.2**
	3	No preparations	408.9	30.8	93.3	533.0
		With preparations	462.4	29.6	124.3	616.4
Fresh manure 25 ton/ha	1	No preparations	504.7	54.4	78.2	637.3
		With preparations	414.3	41.3	55.9	511.5
	2	No preparations	575.5	63.3	126.8	786.5
		With preparations	669.6	66.0	127.2	876.0
	3	No preparations	418.6	34.1	87.9	540.6
		With preparations	402.4	29.2	104.5	536.2
Compost 50 ton/ha	1	No preparations	415.1	46.1	63.2	537.0
		With preparations	470.7	45.4	70.4	632.6
	2	No preparations	590.6	50.3	108.4	749.3
		With preparations	631.1	56.4	118.1	805.6
	3	No preparations	388.6	25.9	110.6	525.1
		With preparations	383.3	30.2	101.6	515.1
Fresh manure 50 ton/ha	1	No preparations	477.3	52.5	73.4	615.0
		With preparations	474.7	55.9	64.9	628.3
	2	No preparations	529.3	46.9	101.8	678.0
		With preparations	602.5	56.2	130.9**	810.4**
	3	No preparations	417.1	29.0	101.8	547.9
		With preparations	363.9	25.5	79.2	468.7

Quality indices

Sampling was made three times, 24th of September 2006, 23rd of September 2007 and 20th of October 2007 in all treatments, total number of samples = 136. Besides the regular analysis when calculating the quality indices according to Pettersson also analysis of dry matter content, amount of ascorbic acid, amount of organic acids and amount of nitrate was performed.

During 2006 the trial was manured according to plan while during 2007 only plots with chicken manure were fertilized. Comparing the results from the three sampling dates gives the following differences:

Table 26. Mean values of all samples at different sampling dates. Numbers followed by the same letter is not significantly different, P < 0.05.

Sampling date	N	Dry matter	Ascorbic acid	Nitrate	Total sugar	Organic acids	Free amino acids	Extract combustion	Quality indice
24.09.2006	40	9.80 b	64.95 a	262.25 a	8.53 c	1.02 b	347.15 a	19.37 a	92.50 c
23.09.2007	48	10.69 a	53.58 b	63.19 b	9.74 a	1.33 a	176.25 c	10.72 b	131.04 a
20.10.2007	48	9.94 b	54.46 b	51.85 b	9.20 b	0.90 c	316.73 b	10.65 b	113.70 b

Influence of harvest year and harvest period

The two sampling dates in September differs significantly from each other on all analysis. With the exception of the amounts of ascorbic acid all other analysis indicates a higher quality in the carrots sampled in September 2007. Besides seasonal differences this must be explained with the use of manures during 2006. Also the two sampling dates during 2007 differs significantly from each other in most respect with the exception of the amounts of ascorbic acids and nitrate and also the rate of extract combustion. The results indicate a decrease in the quality of the carrots from the sampling in September until the sampling in October 2007.

Influence of type of manure

The type of manure influenced the dry matter content, the amounts of ascorbic acid, nitrate, total sugars, and organic acids. Especially the use of chicken manure influenced the carrots in a positive way, especially during 2007.

Table 27. Mean values of all samples from different types of manure. Numbers followed by the same letter is not significantly different, P < 0.05.

	Dry matter	Ascorbic acid	Nitrate	Total sugars	Organic acids	Free amino acids	Extract combustion	Quality indice
No manure	10.14 b	58.27 b	99.36 ab	9.19 ab	1.05 b	261.95 a	12.47 a	115.64 a
Chicken manure	10.62 a	62.18 a	90.18 b	9.40 a	1.18 a	275.14 a	15.21 a	112.09 a
Fresh stable manure	10.03 b	56.29 b	121.25 ab	9.07 b	1.07 ab	276.13 a	13.97 a	111.71 a
Composted stable manure	10.17 b	57.21 b	146.58 ab	9.25 ab	1.15 ab	269.33 a	12.68 a	114.79 a

Influence of amount of manure

The amount of fresh or composted manure used did not influence the properties of the carrots with the exception of the amounts of ascorbic acid.

Table 28. Mean values of all samples manured with different amounts. Numbers followed by the same letter is not significantly different, $P < 0.05$.

	Dry matter	Ascorbic acid	Nitrate	Total sugars	Organic acids	Free amino acids	Extract combustion	Quality indice
No manure	10.14 a	58.27 a	99.36 a	9.19 a	1.05 a	261.95 a	12.47 a	115.64 a
25 ton/ha	10.01 a	54.77 b	123.05 a	9.13 a	1.03 a	287.32 a	12.54 a	113.67 a
50 ton/ha	10.10 a	56.75 ab	133.92 a	9.16 a	1.11 a	272.73 a	13.33 a	113.25 a

Influence of biodynamic preparations

Seen in general of all samples use of the biodynamic preparation significantly decreased the amounts of sugars in carrots.

Table 29. Mean values of all samples treated or not treated with the biodynamic preparations. Numbers followed by the same letter is not significantly different, $P < 0.05$.

	Dry matter	Ascorbic acid	Nitrate	Total sugars	Organic acids	Free amino acids	Extract combustion	Quality indice
No preparations	10.16 a	57.31 a	111.94 a	9.27 a	1.09 a	274.15 a	13.52 a	112.99 a
With preparations	10.17 a	57.16 a	123.53 a	9.12 b	1.08 a	278.04 a	12.95 a	114.18 a

As can be seen in table 28 there were significant differences between samples treated or not treated with the biodynamic preparations.

Table 30. Mean values of all samples of different treatments. Numbers followed by the same letter is not significantly different, P< 0.05.

Datum	Treat-ment	Dry matter	Ascorbic acid	Nitrate	Total sugars	Organic acids	Free amino acids	Extract combustion	Quality indice
24.09.2006	0+	9.93 b	64.67 ab	253.33 bc	8.77 ab	0.91 cd	320.00 cd	15.47 b	103.33 ab
	0-	9.37 b	65.00 ab	260.00 bc	8.30 b	1.07 bcd	346.67 bcd	18.57 ab	93.67 abcd
	F2+	9.71 b	69.67 a	186.67 b	8.33 b	0.84 d	286.00 d	14.43 b	107.33 ab
	F2-	9.31 b	60.67 b	200.00 bc	8.40 b	0.91 cd	282.00 d	13.57 b	109.33 a
	F3+	9.64 b	65.75 ab	250.00 bc	8.55 b	1.09 abc	400.50 ab	19.55 ab	87.50 abcd
	F3-	9.51 b	67.50 ab	315.00 abc	8.50 b	0.98 bcd	368.75 abc	23.65 ab	82.75 bcd
	H+	11.99 a	64.67 ab	173.33 c	8.40 b	1.16 ab	322.33 cd	19.63 ab	94.33 abcd
	H-	9.41 b	62.67 ab	333.33 ab	8.40 b	0.99 bcd	424.33 a	25.00 ab	71.33 d
	K2+	9.49 b	63.67 ab	256.67 bc	8.50 b	1.01 bcd	342.00 cd	27.23 a	78.67 cd
	K2-	10.18 b	63.33 ab	190.00 b	8.77 ab	1.22 a	363.33 abc	18.73 ab	92.33 abcd
	K3+	9.23 b	67.25ab	402.50 a	8.30 b	1.07 abc	328.25 cd	18.10 ab	96.00 abc
	K3-	10.04 b	63.25 ab	265.00 bc	9.05 a	1.02 bcd	359.00 bc	17.95 ab	96.00abc
	23.09.2007	0+	10.74 abc	49.75 efg	31.75 b	9.60 bc	1.17 bc	174.25 bc	11.76 abc
0-		10.54 bc	54.75 bcd	37.75 b	9.80 abc	1.31 abc	184.50 abc	9.59 bcd	130.50 a
F2+		10.64 abc	56.75 bcd	53.00 b	9.80 abc	1.41 ab	217.75 a	9.84 bcd	134.75 a
F2-		10.43 cd	48.25 fg	81.25 b	9.50 c	1.29 abc	194.75 bc	11.56 abc	125.00 a
F3+		10.70 abc	52.25 def	27.50 b	9.68 bc	1.42 ab	148.50 c	8.60 e	136.00 a
F3-		10.78 abc	54.25 bcde	37.50 b	9.85 abc	1.40 ab	147.50 c	11.13 abcd	130.50 a
H+		11.03 ab	59.00 ab	43.25 b	10.10 ab	1.50 ab	171.25 bc	12.31 ab	135.25 a
H-		11.17 a	62.25 a	28.25 b	10.25 a	1.24 bc	197.00 ab	13.15 a	129.25 a
K2+		9.99 d	46.00 g	220.25 a	9.03 d	0.98 c	180.50 abc	8.88 de	129.75 a
K2-		10.46 cd	49.75 efg	105.75 b	9.70 bc	1.13 bc	179.50 bc	12.28 ab	126.00 a
K3+		10.81 abc	57.25 bc	52.00 b	9.70 bc	1.46 ab	160.50 bc	9.18 de	133.50 a
K3-		10.99 abc	52.75 def	40.00 b	9.85 abc	1.63 a	159.00 bc	10.35 bcde	130.75 a
20.10.2007 I		0+	10.11 ab	56.00 abc	28.25 b	9.20 bcde	0.87 b	278.75 c	10.53 a
	0-	9.89 ab	62.75 a	63.75 b	9.13 bcde	0.92 b	303.25 c	11.20 a	114.25 ab
	F2+	9.91 ab	52.50 bcd	30.75 b	9.20 bcde	0.96 b	383.25 ab	9.65 a	109.38 bc
	F2-	10.32 a	58.75 ab	23.50 b	9.70 a	0.95 b	299.00 c	10.40 a	116.00 ab
	F3+	9.77 bc	51.00 bcd	42.25 b	8.88 de	0.75 b	284.75 c	10.85 a	117.50 ab
	F3-	9.79 bc	47.00 d	55.25 b	8.95 cde	0.75 b	306.75 c	10.03 a	116.00 ab
	H+	10.06 ab	63.50 a	19.75 b	9.33 abcd	0.95 b	301.75 c	13.03 a	111.50 abc
	H-	10.12 ab	61.75 a	24.75 b	9.43 ab	1.17 a	283.25 c	11.73 a	116.25 ab
	K2+	9.39 c	44.50 d	168.25 a	8.80 e	0.87 b	411.25 a	9.73 a	105.50 c
	K2-	10.02 ab	53.00 bcd	45.75 b	9.25 bcde	0.81 b	339.50 bc	10.13 a	113.25 abc
	K3+	10.02 ab	53.25 bcd	78.00 b	9.40 abc	0.90 b	332.75 bc	10.65 a	113.25 abc
	K3-	9.92 ab	49.50 cd	42.00 b	9.20 bcde	0.85 b	276.50 c	9.88 a	119.25 a
	0+	10.29 b	56.09 bc	90.91 c	9.23 ab	0.99 bc	252.00 a	12.32 ab	116.73 a
	0-	9.98 bc	60.45 ab	107.82 bc	9.15 abc	1.10 abc	271.91 a	12.62 ab	114.55 a
	F2+	10.12 bc	58.73 abc	81.36 c	9.18 abc	1.09 abc	296.55 a	11.02 b	118.05 a
	F2-	10.08 bc	55.45 bc	92.64 c	9.27 ab	1.06 abc	256.45 a	11.69 ab	117.45 a
	F3+	10.04 bc	56.33 bc	106.58 bc	9.03 bc	1.09 abc	277.92 a	13.00 ab	113.67 a
	F3-	10.02 bc	56.25 bc	135.92 bc	9.10 abc	1.05 abc	274.33 a	14.94 ab	109.75 a
	H+	10.94 a	62.18 a	70.18 c	9.35 ab	1.21 a	259.91 a	14.57 ab	115.45 a
	H-	10.31 b	62.18 a	110.18 bc	9.45 a	1.15 abc	290.36 a	15.86 a	108.73 a
K2+	9.63 c	50.27 d	211.27 a	8.80 c	0.95 c	308.45 a	14.19 ab	107.00 a	
K2-	10.22 b	54.64 cd	106.91 bc	9.28 ab	1.04 abc	287.82 a	13.25 ab	112.18 a	
K3+	10.02 bc	59.25 abc	177.50 ab	9.13 abc	1.14 abc	273.83 a	12.64 ab	114.25 a	
K3-	10.32 b	55.17 bc	115.67 bc	9.37 ab	1.17 ab	264.83 a	12.73 ab	115.33 a	
Total									

Taste experience

The experience when tasting the carrot samples was summarized into five categories.

- Woody: An experience of a carrot hard to swallow, leaving a unpalatable root part in the mouth
- Juicy: A carrot rich in fluids
- Sweet: A carrot awaking a sensation of sweetness
- Bitter: A carrot awaking a heavy and astringent sensation on the back of the tongue
- Harsh: A carrot awaking a burning sensation similar to turpentine

Each notation with a category was given the value 1. The figures shows the sum of notations. Total number of samples = 365.

Influence of harvest year and harvest period

The harvest season had a major influence on the taste of carrots. During the year of 2006 the carrots became more bitter and to some extent also sweet whereas harsh, woody and juicy tastes was not so well established (**Figure 36**). The year of 2007 is gave more juicy or woody carrots and the year of 2008 more sweet, bitter or harsh tasting carrots (**Figure 36**).

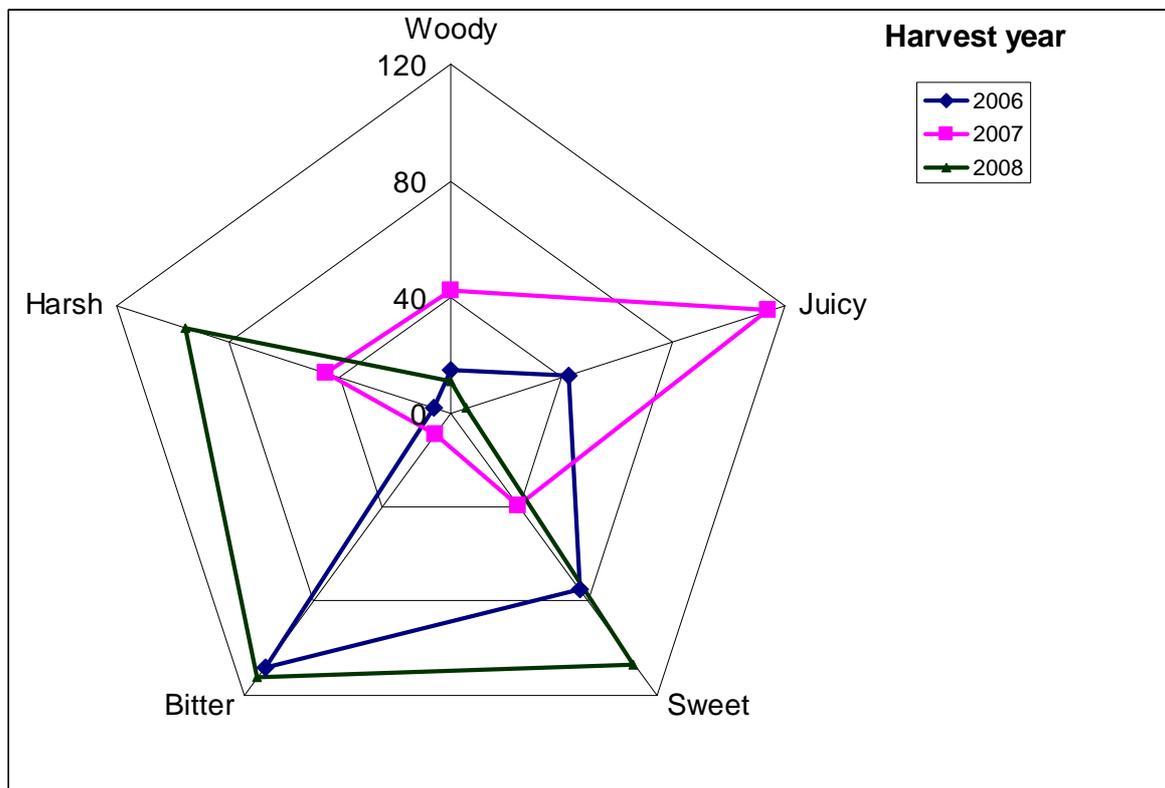


Figure 66. Profile of taste experiences in carrots. Sum of all samples 2006, 2007 and 2008. N=365

Carrots harvested very early, at harvest number 1 had a smaller expression of tastes than carrots harvested later (**Figure 37**). Juicy, harsh and sweet tasting carrots were more common when the carrots at harvest number 2 and bitter tasting or woody carrots during harvest number 3 (**Figure 37**).

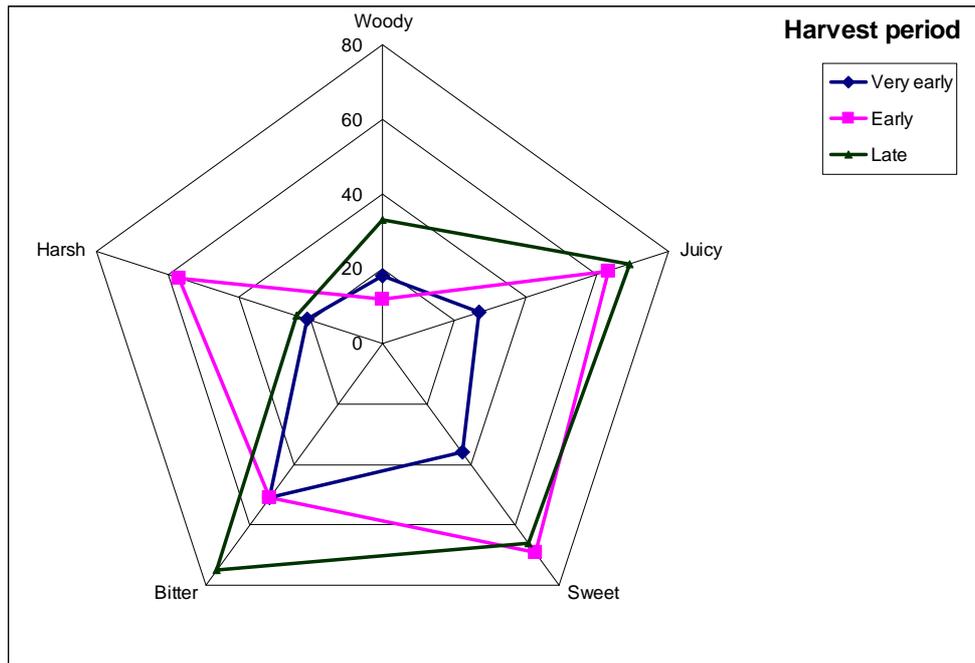


Figure 67. Profile of taste experiences in carrots. Sum of all samples from different harvest periods 2006, 2007 and 2008, N=365

Influence of type of manure

When unmanured harsh tasting carrots were more common, among carrots manured with chicken manure sweet taste was common whereas composted manure lead to more juicy carrots (**Figure 38**).

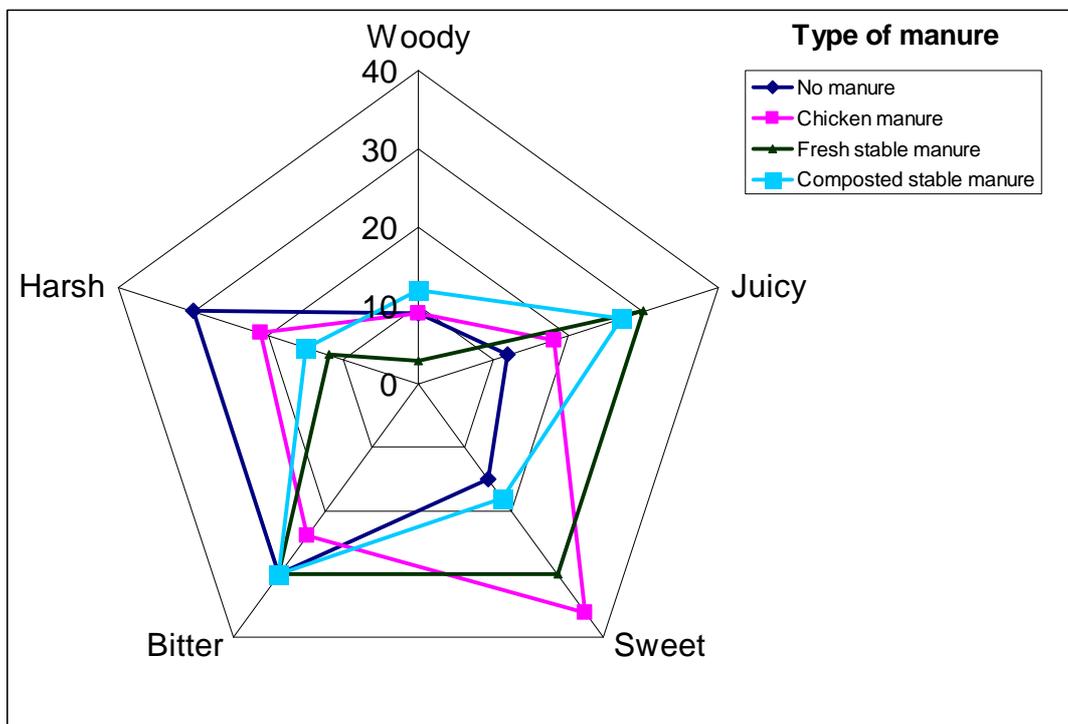


Figure 68. Profile of taste experiences in carrots. Sum of samples cultivated in different type of manures 2006, 2007 and 2008., N=365

Influence of amount of manure

The richest taste spectrum was found in carrots manured with 25 tons of fresh or stable manure per hectare (**Figure 43**). Carrots grown in unmanured soil had a small taste spectrum especially concerning harsh or sweet taste and carrots grown in soil manured with 50 tons had a spectrum towards more harsh and not so juicy carrots (**Figure 39**).

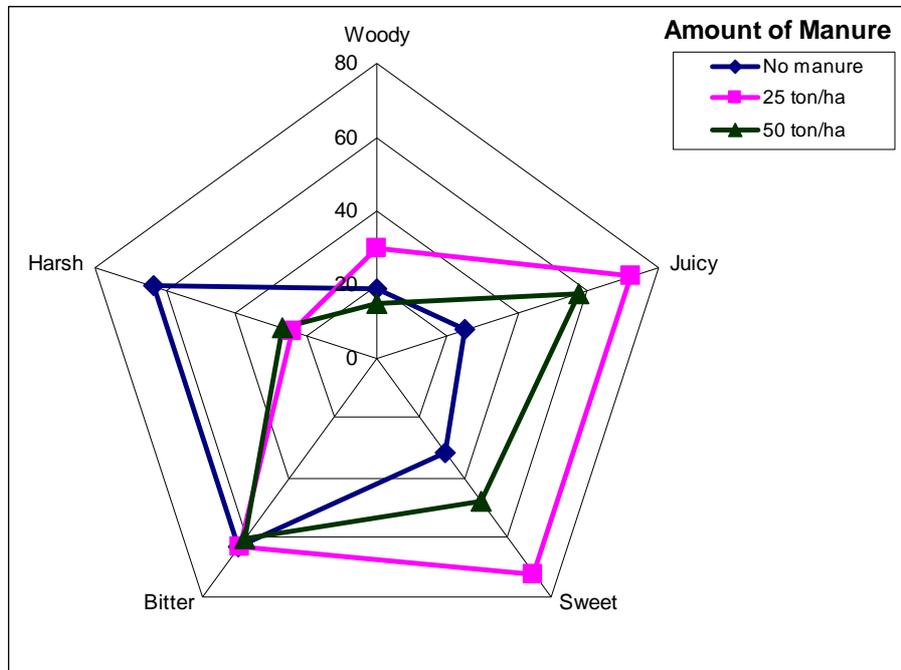


Figure 69. Profile of taste experiences in carrots. Mean of samples cultivated with different amounts of fresh or composted stable manure 2006, 2007 and 2008, N=365

Influence of biodynamic preparations

In general the biodynamic preparations did not effect the taste of the carrots (**Figure 40**).

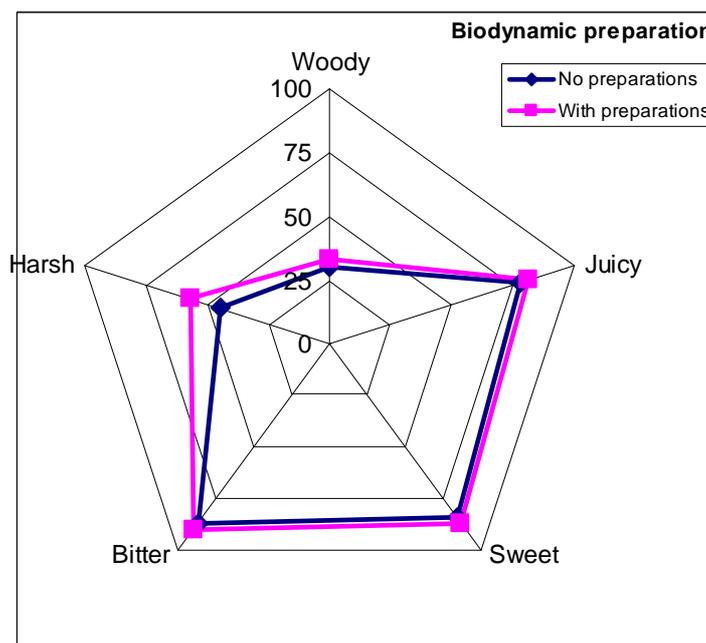


Figure 70. Profile of taste experiences in carrots. Sum of samples untreated of treated with the biodynamic preparations 2006, 2007 and 2008, N=365

When expressing the taste spectra in the different fertilising systems differences occurred between samples treated or not treated with biodynamic preparations (**Figure 41**). There was however hard to find a pattern in these differences.

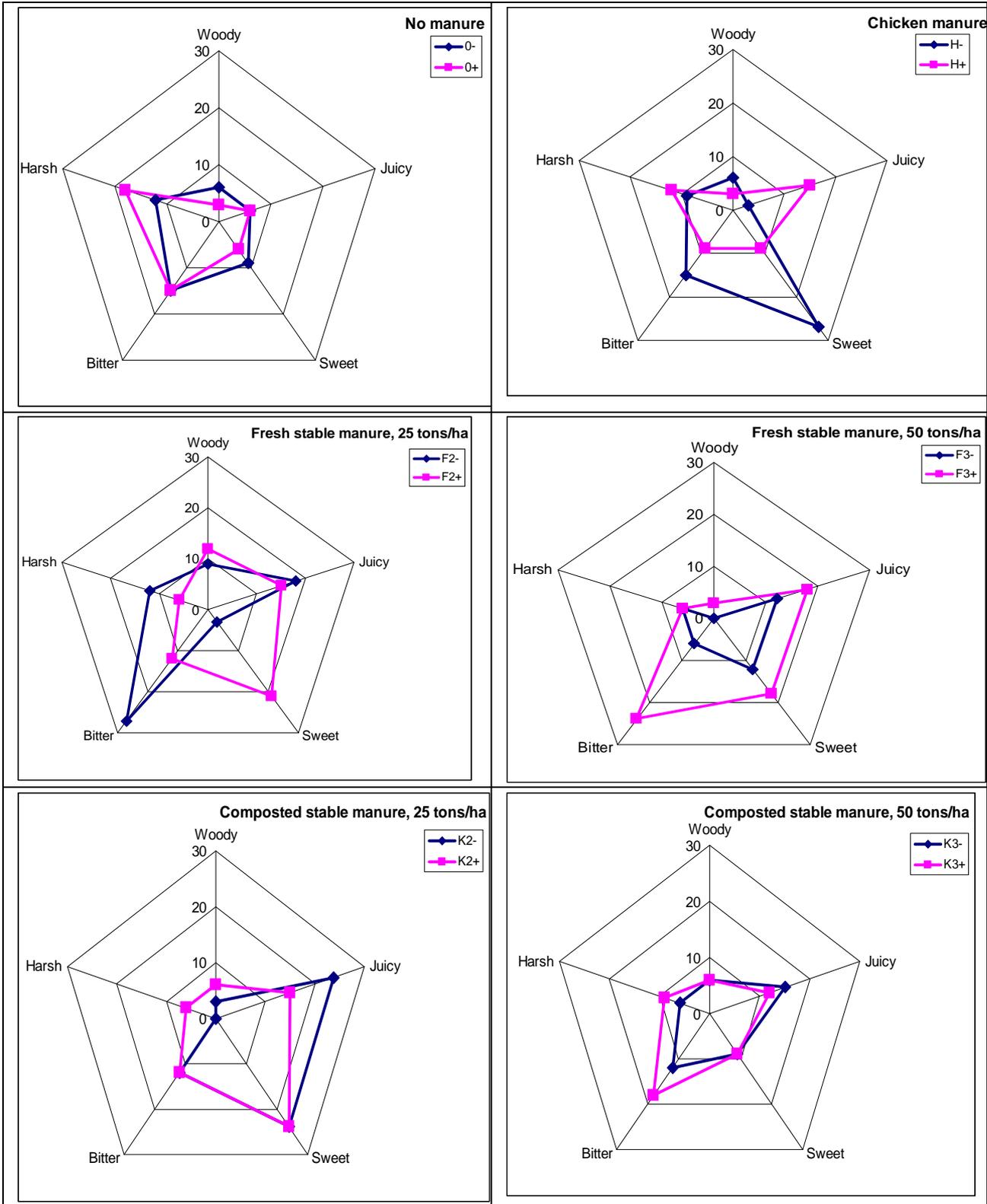


Figure 71. Profile of taste experiences in carrots. Sum of samples cultivated under different fertilisation regimes 2006, 2007 and 2008

Picture forming methods

Table 31. Thumbnails of biochrySTALLISATION pictures and biochromatogrammes . Full size picture are available on attached DVD or by clicking on the thumbnail.

Treatment	Harvest number 1	Harvest number 2	Harvest number 3	Soil
No manure, no prep				
No manure, BD prep				
Chicken manure, no prep				
Chicken manure, BD prep				
Fresh 25 tons, no prep				
Fresh 25 tons, BD prep				
Fresh 50 tons, no prep				
Fresh 50 tons, BD prep				
Compost 25 tons no prep				
Compost 25 tons, BD prep				
Compost 50 tons, no prep				
Compost 50 tons, BD prep				

The soil

Three soil samples were taken from each parcel at a depth of 20-25 cm. The soil was dry, hard and packed, due to lack of sufficient precipitation.

The samples from parcel 801, harvest 1 2008, fresh manure, 25 tonnes with biodynamic preparations are here used as a standard when comparing pictures from different treatments



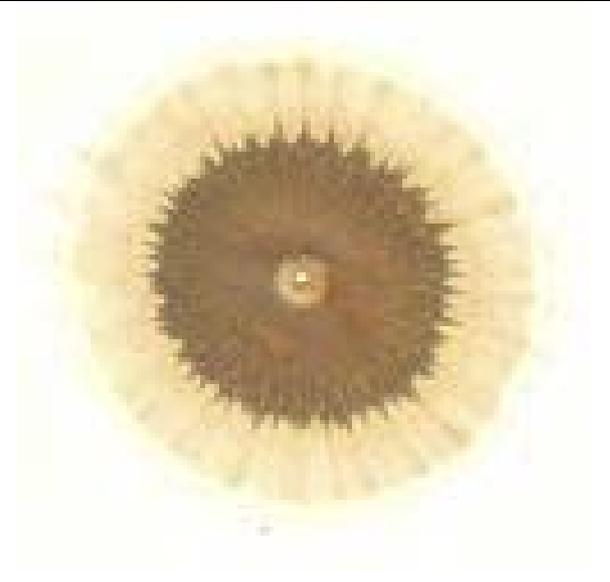
Figure 72. BiochrySTALLISATION picture from plot 801 used as a standard

Influence of type of manure

Soil:

All samples indicate a healthy and well balanced soil. The pictures have clear to bright yellow-brown colours, and distinct contiguousness between the zones. No manure and chicken manure gives a more solid and heavy impression, whereas fresh and composted manure gives a more detailed and lighter impression in comparison.

Table 32. Biochromatogrammes of soil samples

	
No manure no BD prep	Compost manure 25 tonnes BD prep

Carrots:

The majority of the samples have structured needle formations, in most cases a typical root formation in the centre. The difference between the manures indicates that fresh and composted manure gives the overall best structured pictures in all three harvests.

Influence of amount of manure

Soil:

Compared to the more solid pictures in no manure and chicken manure, the fresh 50 tonnes and compost 25 and 50 tonnes, indicates a raise in soil activity showing as bright yellow-brown colours, and distinct contiguousness between the zones.

Carrots:

The majority of the samples has structured needle formations, and in most cases a typical root formation in the centre and branches all the way to the edge zone. The amount of manure indicates that fresh and composted manure gives the overall best pictures, except for the 50 tonnes. These pictures have a tendency to be without the typical root formation in the centre and/or coalesced, without distinct branches to the edge zone.

Influence of biodynamic preparations

Soil:

All samples indicates a balanced, healthy and active soil and the differences derives from the type and amount of manure. The fresh manure with BD prep and compost manure with BD prep show a clear increase in soil activity compared to the others. The yellow-brown colors are more vivid and the distinct contiguousness between the zones is more detailed.

Carrots:

In comparison with no BD preparations there is a tendency in these pictures, to overcome the coalesced extract and reach a state of equilibrium. Depending on the amount of manure, this has a varied degree of success. Increasing amounts of manure gave rise to more distortion in the pictures. BD prep seems to support the establishment of the needle structures in the overall pattern, but without an accurate microscopic test, this can not be verified at this stage.

Correlations between methods

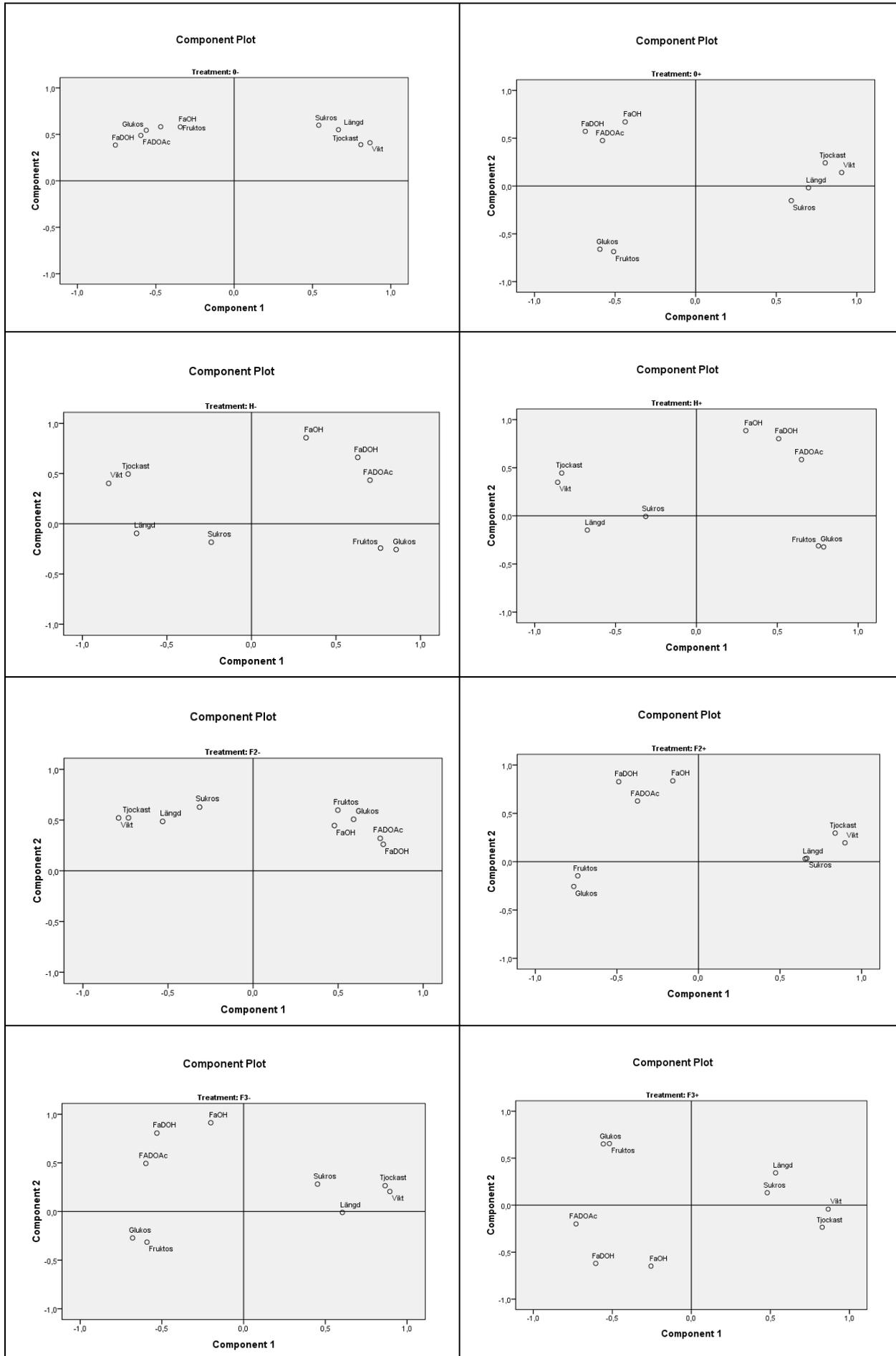
The different methods used when trying to describe the properties of the carrots were chosen in order to enlighten different sides of carrot quality. Falcarindiol and falcarindiol-3-acetate were inversely correlated to root size and the amounts of sucrose, but positively correlated to the amounts of hexoses (**Table 33**). There were also strong negative correlations between the amounts of all polyacetylenes and the amounts of free amino acids (**Table 33**). The amounts of hexoses and the quality indices according to Pettersson were inversely correlated to root size, whereas the amounts of sucrose exhibited a positive correlation to root size (**Table 33**). Root size was negatively correlated to the content of dry matter but positively correlated with the combustion rate of extracts and with the amounts of free amino acids (**Table 33**).

Table 33. Correlation coefficients between results from different analysis of carrot roots. ** Correlation is significant at the 0.05 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Squares marked yellow indicate a significant positive correlation

while purple squares indicate a significant negative correlation. Columns are shaded differently according to the groups of analysis.

	FaDOH	FaDO-Ac	FaOH	Fructose	Glucose	Sucrose	Weight	Length	Thickness	DM	Asc. Ac	Nitrate	Sugar	Org. Ac	Free Amino	Combustion	Indice
FaDOH	1																
FaDOAc	.667**	1															
FaOH	.681**	.439**	1														
Fructose	.164**	.276**	.029	1													
Glucose	.212**	.342**	.013	.928**	1												
Sucrose	-.131**	-.134**	.016	.035	.032	1											
Weight	-.234**	-.323**	.031	-.303**	-.409**	.456**	1										
Length	-.313**	-.099**	-.178**	-.088**	-.098**	.403**	.586**	1									
Thickness	-.160**	-.328**	.120**	-.327**	-.464**	.450**	.943**	.434**	1								
Dry Matter	.197**	.229**	.035	-.003	.025	.049	-.302**	-.157**	-.334**	1							
Ascorbic Ac	-.151**	-.174**	-.128**	.025	-.100**	-.227**	.338**	.050	.378**	.111*	1						
Nitrate	-.127**	-.193**	-.054	.249**	.153**	-.359**	.203**	-.076	.277**	-.471**	.210**	1					
Sugar	.322**	.349**	.137**	-.117**	-.039	.117*	-.410**	-.109*	-.486**	.640**	-.045	-.665**	1				
Org. Ac	.345**	.322**	.055	-.020	-.010	-.219**	-.401**	-.279**	-.402**	.269**	.093	-.105*	.387**	1			
Free Amino	-.497**	-.480**	-.251**	-.124**	-.213**	-.058	.599**	.361**	.646**	-.544**	.167**	.517**	-.542**	-.261**	1		
Combustion	.122*	.060	.108*	.073	-.042	-.217**	.256**	-.020	.330**	-.187**	.287**	.228**	-.215**	.020	.332**	1	
Indice	.197**	.230**	.060	-.005	.118*	.168**	-.500**	-.173**	-.578**	.455**	-.267**	-.461**	.516**	.145**	-.739**	-.830**	1

When applying factor analysis on the results it turns out that the variation of the different methods used behave a little differently depending on the fertilizing treatments used (**Figure 69**). As each component plot is calculated separately the different components differ slightly from each other. Therefore it is the proximity between different analysis that is important, not their position in the coordinate system. The unmanured treatment, not treated with the biodynamic preparations, 0-, is used as a reference when describing the differences between the treatments. In 0- there are two groups, one with the polyacetylenes and the hexoses, the other consisting of sucrose and root size. When fertilizing with chicken manure, H-, the plot exhibits four groups, polyacetylenes, hexoses, root weight and thickness and finally root length and also although slightly separated root length and sucrose. Carrots treated with 25 tons of fresh manure, F2-, are similar to the unmanured plot with two distinct groups whereas samples manured with 50 tons of fresh manure exhibits three groups, slightly similar to H- but with sucrose and root length closer to root weight and root thickness. When treated with 25 tons of composted manure, K2-, there are three distinct groups with sucrose and root length closer to root weight and root length than in F3-. Finally when manuring with 50 tons of compost, K3-, there are two not so distinct groups, as faltarinol, sucrose and root length forms a unit in between root weight and root thickness on the one side and the hexoses and the faltarindiols on the other side. Between plots treated or not treated with the biodynamic preparations there were differences in the pattern of variance. In the unmanured plots and in the pots manured with 25 tons of fresh manure the biodynamic preparation contributed to a pattern of variance more similar to the highest manured plots with the hexoses distinctly separated from the polyacetylenes, 0+. In the treatments F3 and H there were no significant differences in the pattern of variance if the biodynamic preparations were used or not. In K2 treatment with the biodynamic preparations brought the hexoses closer to the polyacetylenes, as it made it more similar to an unmanured situation whereas in K3 the biodynamic preparations influenced to a situation where the variance more distinctly separates the polyacetylenes, hexoses and root size and sucrose (**Figure 69**).



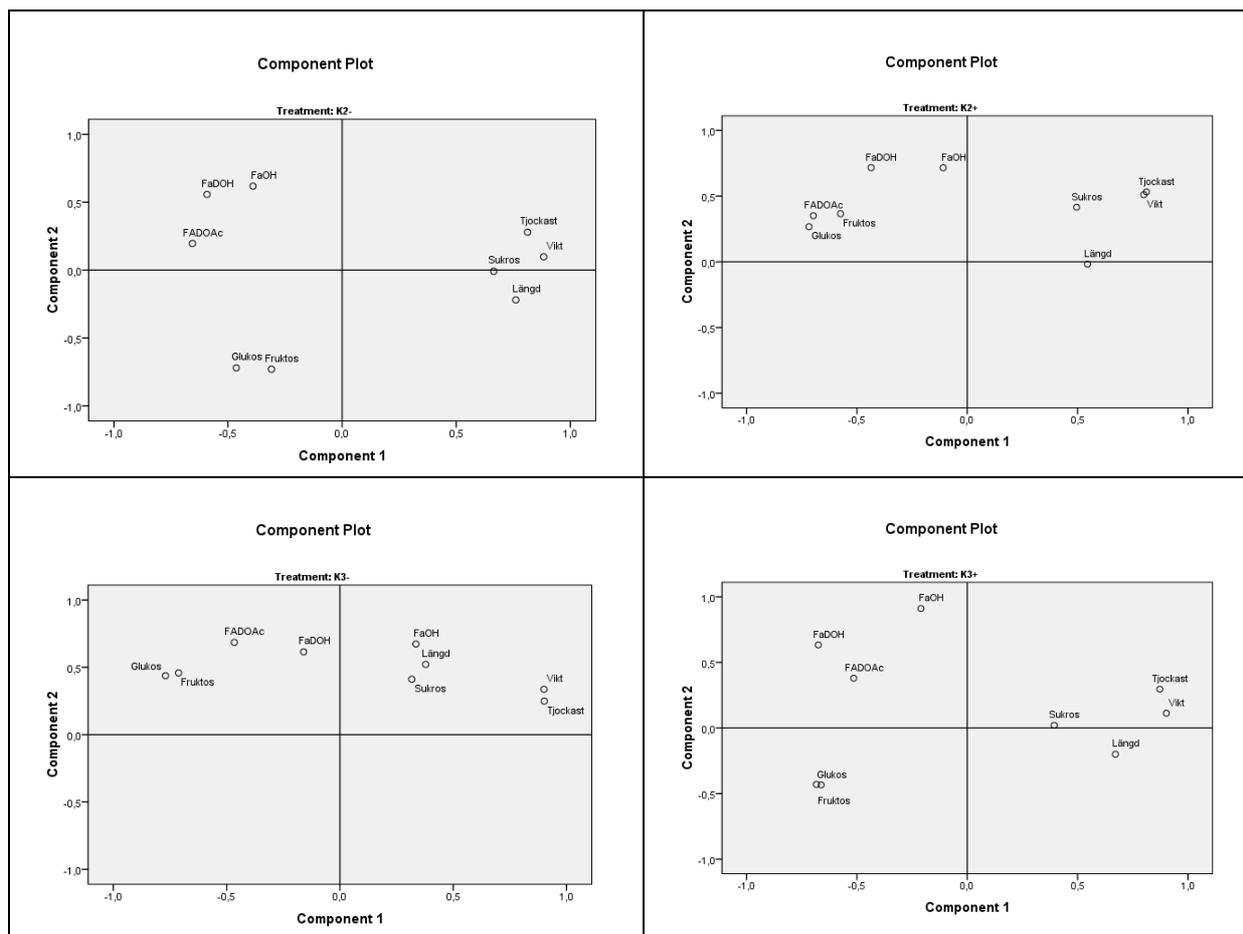


Figure 73. Principle component analysis, applied to the different treatments in the field trial. Results from analysis in carrots 2006 to 2008, Vikt= Weight, Längd=Length, Tjockast=Thickness.

Discussion

Organic agriculture has attained an increased attention during the past decades. The simplest definition on organic agriculture is the avoidance of easy soluble synthetic fertilizers and chemical pesticides. This characterization emphasis on a methodological level by describing what is done or what is not done. Another way to describe organic agriculture is to emphasis on a more functional level by characterizing organic agriculture as a system. Within the framework of organic agriculture there is thus a wide variety of methods and systems to choose from when producing an organic carrot. The use on a farm of imported pelleted chicken manure represents an agriculture influenced by the same thinking as in conventional agriculture. Here this way of farming will be referred to as the industrial organic agriculture, IOA. Recently the term ecological recycling agriculture, ERA, has been introduced¹⁹³. The term is used to describe an organic agriculture striving towards farm self-sufficiency and recycling with local resources. A third form of organic cultivation is the biodynamic agriculture, BDA, that differs from ERA mainly by the use of special compost and field preparations^{194, 195}.

According to the results presented here manuring strongly influences the properties of the soil, but the properties of the crops are not so strongly influenced, not even after a 21 year experiment period. This confirms similar results from reports concerning other long term field experiments^{2, 7, 196-216}. When evaluating different factors influencing the properties of the crop geographic, climatic, annular and seasonal variation seems most important followed by the choice of variety and thereafter by the different cultivation measures². The biological properties of the soil, such as number of earthworms and activity of microbes is strongly influenced by the manuring systems^{2, 200, 202, 204, 207, 213, 215, 217-219}.

The plant is however more obviously influenced by the physical properties of the soil than by the biological properties. This become evident when comparing carrots harvested 2006 with carrots harvested 2008 (**Figure 74**). The year of 2008 offered a hard layer in the soil at approximately 10-15 cm depth. The carrots had difficulties in penetrating this layer. Instead they grew thicker in the upper end, and the root end was often split up into two or more root endings. As the roots were having difficulties in growing into the soil, the hypocotyl part of the carrot instead expanded upwards, resulting in plenty of green necks. Together with a very dry first part of the season where the carrots had to be sown again as late as in the middle of June the carrots harvested during 2008 differed strongly from carrots harvested during 2006 or 2007.



Figure 74. Comparison between carrots harvested 2006 (left) and 2008 (right), explanation in text. Variety 'Kämpe'.

As can be seen from the example the carrot plant has a strong ability to adjust its growth according to the situation at the growing site. So why does not the biological properties of the soil influence the pattern of growth more distinct? One reason to this might lay in the genetical prerequisites of the seeds. An example to illustrate this is shown in figures 75 to 80. Two varieties of oats were sown side by side in two different types of soils and also with a five week variation in the sowing dates. The development of the plants was followed during the whole growing season. The illustrations show the plants in a series with a distance of approximately 10 days in time. The variety 'Stormogul' shows a broad register of reactions on the different situations offered. It has the ability to react both with an increase in vegetative growth in leaves (**Figure 77**) and in the number of straw (**Figure 75**) or to develop faster towards maturity of the seeds under more generative circumstances (**Figure 76**). This is a variety more open to the external factors surrounding it.



Figure 75. The development of the oat variety 'Stormogul II' cultivated in a soil rich in humus and sown at a normal date.



Figure 76. The development of the oat variety 'Stormogul II' cultivated in a sandy soil and sown at a normal date



Figure 77. The development of the oat variety 'Stormogul II' cultivated in a soil rich in humus and sown five weeks later than the plants in figure 71.

The variety 'Sol II' exhibits a different expression. The development of the plants does not differ so much. The vegetative expressions of the plants are much weaker than by 'Stormogul II'. The plants strive strongly towards maturity of the seeds more or less regardless of the circumstances under which it grow. The plants of 'Sol II' exhibit more the generative properties of oats.

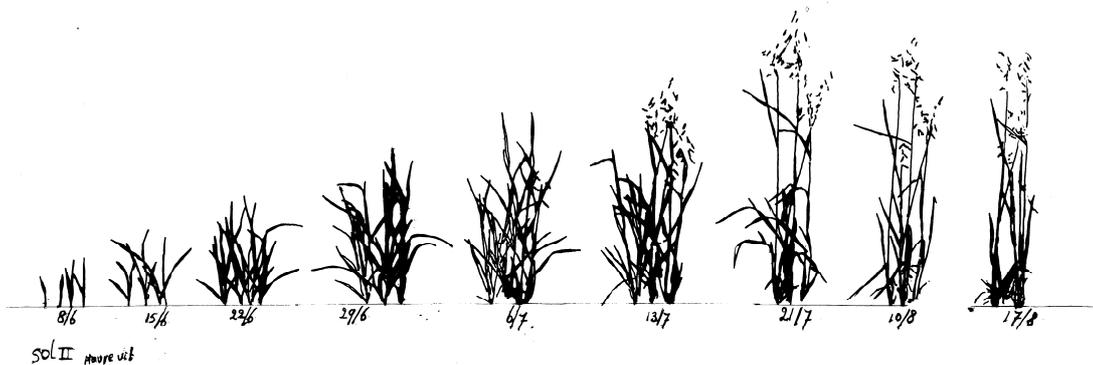


Figure 78. The development of the oat variety 'Sol II' cultivated in a soil rich in humus and sown at a normal date.

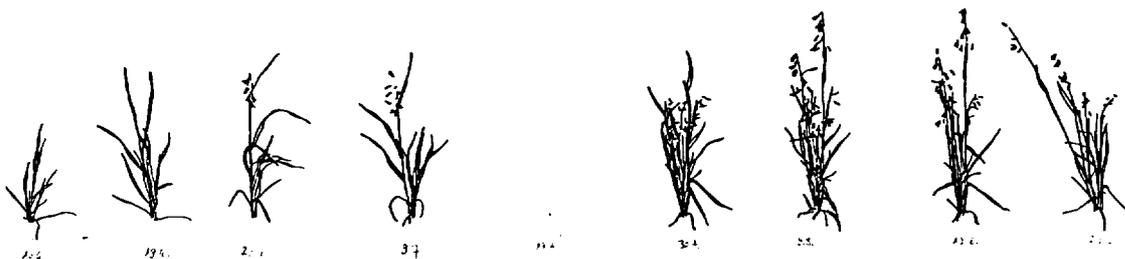


Figure 79. The development of the oat variety 'Sol II' cultivated in a sandy soil and sown at a normal date



Figure 80. The development of the oat variety 'Sol II' cultivated in a soil rich in humus and sown five weeks later than the plants in figure 74.

Due to the legislations, a new variety has to differ from its precursors by having characteristics that are stable and that distinguish it from other varieties. As a consequence of this many varieties on the market today have only weak abilities to react on the more subtle circumstances under which they develop. Then transition period of a soil from a conventional to a biodynamic state is approximately seven years². It seems likely that seeds also need a transition period in order to adapt to the situation on the farm. However during the period of the Skilleby trial no such adaption was propagated so far. One explanation to why the biological properties of the soil are so poorly reflected in the properties of the crop might lie

in the fact that new seeds were bought in and used every year. When comparing different farming systems in the future this factor has to be taken into consideration.

In order to discuss the results concerning the properties of carrots in more detail some background is needed. The length of the carrot root is correlated to the intensity of the light while the thickness is correlated to the amount of warmth^{35, 74, 134, 139, 220-222}. Regarding soluble sugars the development of a carrot crop is often divided into three phases: During the first no soluble sugar is stored, in the second phase only reducing sugars, hexoses, are stored and in the third phase mainly sucrose is stored in the taproot¹²¹. Sugars are transported from the leaves to the tap root mainly as sucrose²²³. The sugars are mainly stored in vacuoles in the parenchymatic tissues^{16, 17}. The total sugar content does not differ much between different parts of the carrot⁷⁴ but the amount of sucrose is higher in the upper part and in the phloem while hexoses, especially fructose, are more common in the centre, in the tip part of the carrot root and in the xylem^{36, 74}. The accumulation of sucrose in the taproot seems to be more influenced by environmental factors than the storage of reducing sugar⁷⁴. In the tap root the hydrolysis of sucrose into the hexoses fructose and glucose is catalyzed by at least two groups of enzymes; invertase and sucrose synthase⁴⁶. The increasing sucrose to hexoses ratio during the harvest season indicate a declining activity of the sucrose-cleaving enzymes in carrots^{20, 21, 23, 47} leading to an accumulation of sucrose in the tap root⁴⁶. A high acid invertase activity has been found in rapidly growing tissues⁴⁷ using hexoses as a source of energy. A high sucrose to hexoses ratio is reported as a sign of physiological maturity in the carrot root¹⁹⁻²⁴.

Using the unmanured situation as a reference the following can be said about the influence of the different forms of manure used in the experiment:

Pelleted chicken manure increased the weight and thickness but not the length of the carrots. It increased the root cylindricity and the amounts of sucrose during the early part of the harvest season but decreased the amounts of hexoses later in the season. The use of pelleted chicken manure decreased the amounts of polyacetylenes in the carrots, but increased the dry matter content and the amounts of ascorbic and organic acids.

In relation to the unmanured samples the use of fresh stable manure increased root weight and during the mid part of the harvest season also the root length. It did not influence the amounts of soluble sugars, polyacetylenes, ascorbic or organic acids. Neither did it influence the dry matter content or the root shape.

Composted manure increased root weight and during the first two harvest also root length. It also increased root cylindricity both early and late in the harvest season. At the first harvest samples manured with compost exhibited higher amounts of sucrose but otherwise there were no difference in the amounts of soluble sugars and polyacetylenes.

A comparison between the different types of manures shows that the pelleted chicken manure increased the size of the carrots but to the cost of a dilution of hexoses and polyacetylenes. This effect could not be detected when using fresh or composted manure where the roots grew bigger without a decrease in nutrients. The high amounts of sucrose but low levels of total soluble sugars in samples manured with chicken manure indicate a change in the enzymatic activity when using this type of manure. This can be seen as a sign of a retardation in the metabolic activity of the carrots roots, especially as the synthesis of secondary substances such as polyacetylenes are low.

Differences between the treatments were more frequent at the second harvest. Both earlier and later the samples were more equal regardless of treatment. This indicates differences in the growth pattern and in the rhythm of development and maturity. However in order to study this more in detail a more frequent sampling is needed.

The biodynamic preparation also seems to influence the growth pattern of the carrots. It has been suggested that the biodynamic preparations should have a regulating effect on the growth of the crops, increasing low levels and decreasing high²²⁴. It must however be stressed that significant effects of the biodynamic preparation are not so frequent and that it statistically is more plausible with a significant difference when one value is extreme. The influence of the biodynamic preparation on the quality of the crop seems to be on a complex level. It thus challenges our ability to think in context rather than to analyse in details. This hypothesis is confirmed by the results in this report, not least by the principal component analysis and the picture forming methods.

The different treatments in the Skilleby field experiment concerns fertilizing, mainly with different kinds of manure. These are applied within a fixed system of crop rotation, soil management and other cultivation methods performed on the farm. The Skilleby field experiment thus makes it possible to compare different manuring regimes and also different manuring systems as part of a farming system. The manuring regimes consist of the 12 different treatments. In order to relate the results from the field experiment to different forms of organic agriculture four different fertilizing systems has been collected. The first is an unmanured fertilizing system, UNM, using the result from the unmanured treatments without biodynamic preparation, 0-, as a reference. The second is the industrial organic agriculture, IOA, using the results from samples treated with pelleted chicken manure without any biodynamic preparation, H-, as a reference. The third is a system with fresh manure from the farm, ERA, using the results from samples manured with 50 tons of fresh manure without any biodynamic preparations, F3-, as reference. The fourth is a biodynamic system, BDA, using samples fertilized with 25 tons of composted manure plus the biodynamic preparations, K2+, as a reference. The treatment with 25 tons of compost was chosen as almost approximately 40% of the weight of the manure is lost during the process of composting. When comparing these four systems it must be kept in mind that they are performed in a 5 year crop rotation applied with the aim to create an ERA farm and the situation is therefore not fully representative to IOA

Table 34. Differences between manuring systems on the properties of carrots, 2nd harvest, mean of samples from 2006 and 2007.

Manuring system	N	Weight	Fructose	Sucrose	Poly-acetylenes	Ascorbic acids	Free amino acids	Combustion of extract
UNM	24	45.0 b	143.8 ab	157.8 ab	860.3 a	54.8 b	184.5 ab	9.6 bc
IOA	24	58.3 a	141.4 b	173.8 a	649.9 b	62.3 a	197.0 a	13.2 a
ERA	24	58.5 a	157.9 a	143.6 b	686.9 b	54.3 b	147.5 c	11.1 b
BDA	24	53.2 ab	139.0 ab	168.2 ab	760.8 ab	57.3 ab	160.5 bc	9.2 c

Considering the results in table 34, with samples from the second harvest, an UNM system gives smaller carrots, high levels of polyacetylenes and free amino acids but low amounts of ascorbic acids and slow combustion of extract. Carrots from an IOA manuring system are big, have low amounts of fructose and polyacetylenes, high levels of sucrose, ascorbic and free amino acids and exhibit a rapid combustion of extract. The amounts of polyacetylenes have been reported to decrease in bigger carrots¹²⁹. An ERA system gives big carrots with high amounts of fructose, low amounts of sucrose, polyacetylenes, ascorbic and free amino acids and a medium stable extract. Carrots from a BDA system medium sized with medium

amounts of fructose, sucrose, polyacetylenes, ascorbic and free amino acids and the extract is very stable. Polyacetylenes in carrots has been reported to be inversely correlated to root size¹²⁹. The low amounts of polyacetylenes in IOA and ERA samples are therefore probably a result of “dilution” that are not so strong in UNM. The carrots from the BDA manage to find a balance between root size and the amounts of polyacetylenes. A higher sucrose to hexoses ratio has been reported as a sign of carrot maturity¹⁷ and the differences between the IOA and the ERA probably reflects differences in the maturity of the carrots at time of harvest. Samples from IOA seem to mature more rapidly than carrots from ERA. Also considering this aspect the BDA carrots hold a position between the two other manuring systems. The more balanced chemical composition among the BDA carrots is also reflected in the very stable extract exhibiting a good ability to withstand combustion.

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