Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Fakulteten för landskapsplanering, trädgårds– och jordbruksvetenskap**

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– Interaction with bioactive compounds, socio-economic effects of *E.coli* outbreak in food system

## Tarmsmittor på frukt och grönsaker

– Samspel mellan bioaktiva substanser och *E.coli* samt socio-ekonomiska effekter av utbrott orsakat av *E.coli* i livsmedelskedjan

*Neda Hooshang*


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## Foreword

Being an agroecology student for two years helped me to know more about the idea of sustainability in agriculture. Agroecology helped me to get familiar with the concept of "harmony" between humans and the rest of ecosystems. I learned how it is possible to protect the natural resources with focus on agroecology in practice. I became familiar with organic, alternative, environmentally friendly agriculture through biological pest management or soil fertility management. In general what I have learnt was the study of the ecology of the whole food systems like ecologic and socio-economic aspects. As my background was agriculture and I didn't know anything about social aspects, studying agroecology helped me to learn social science and also more important was to how relate natural science to social science. I learnt how to look at the social aspects of a natural problem in a food system and to analyse the problem from both social and natural perspective. Furthermore, I became able to learn how to define a project and how to proceed in a project step by step. Afterward when I did a research project on *E. coli* in the third semester, my focus went beyond the field and on to the whole food system. So in my thesis I tried to look at EHEC outbreak from broader perspective and link both social and natural areas. In order to do that I wrote a proposal which it included both lab work and social work. Both quantitative and qualitative methods were used in the process of thesis. So in my thesis I tried to use the skills and knowledge which I learnt in agroecology program.

Many thanks to my supervisor and co-supervisor for their helpful advices and guidance throughout the thesis work. Also thanks to my family and friends around the world for their moral support.

## Abstract

The purpose of this study was to investigate the effect of bioactive compounds in the propagation of verotoxin producing *E. coli* using ascorbic acid as a model compound and to identify the socio-economic effects that *E. coli* outbreak have had on vegetable producers, retailers and consumers. The study was done in two parts; laboratory experiments and interviews. One hundred consumers in "Coop" and "Willys" and food quality managers of "ICA", "SABA" and "Grönsaksmästare" participated in the study. Close-ended questions were prepared for consumer's survey. Open-ended interviews were conducted with food quality managers. The laboratory studies revealed that bacterial growth occurred only in 10 mM concentration of ascorbic acid (tested levels: 0.1 mM, 1mM, 10 mM, 100mM). Also bacterial growth in the presence of leaf lysate of three leafy vegetables was proved. Furthermore the consumers' surveys indicated that 84% of respondents were aware of the EHEC (enterohaemorrhagic *E. coli*) outbreak. But 64% of consumers were not affected in their food habits by the EHEC outbreak's news. Interviews with food quality managers revealed that all three companies have been affected economically by the EHEC outbreak in 2011. Also the interviewees pointed out that the role of media, authorities, and consumer responsibility is important in the time of outbreak. The information gap between the authorities and suppliers can damage both producers and retailers. In case of clear evidence of the interaction between bioactive compounds and EHEC, applying different cultural management like selection of leafy vegetables with low bioactive compound content in areas with questionable sanitary irrigation water could be addressed. As a future study studying the interaction of other bioactive compounds with different strains of verotoxin producing *E. coli* is suggested. Furthermore in a broader perspective more collaboration between authorities, media, industry, and academia is necessary.

# 1. Introduction

Food-borne diseases have been a real problem in both developed and developing countries in recent years and, in addition to human pain, have caused major economic losses. It has been proved that in industrialized countries almost one third of the population is affected by food-borne diseases each year, and the problem in developing countries is probably more (FAO/WHO, 2005). In developed countries the data indicates 6 to 81 million cases of illness (Alzoreky et al., 2002).

According to Fan et al., (2009) "the total number of cases of foodborne illnesses in the United States has been estimated to be approximately 76 million illnesses per year, associated with 325,000 hospitalization and 5,000 deaths."

In fact among all the crops, leafy vegetables present the highest concern regarding microbiological hazards at the moment. They have been connected with numerous epidemics with a high number of illnesses in at least three regions of the world and are grown and processed in diverse ways, like pre-cut and bagged products (FAO/WHO, 2008).

Only *E. coli* O157:H7 has caused illnesses in more than 30 countries in different continents. But the reported infections are frequently in Canada, the United States, Japan and the whole of Europe (Bach et al., 2006).

More recently, foodborne outbreaks specifically linked to leafy vegetables were studied in the USA by Herman, Ayers and Lynch (2008). Between 1973 and 2006, 502 (4.8%) outbreaks, 18 242 (6.5%) illnesses and 15 (4.0%) deaths were related to "leafy greens": vegetables such as lettuce, cabbage, mescaline mix, spinach or a salad which contained one or more of these leafy vegetables (FAO/WHO, 2008).

The aim of this study was to see if there is an interaction between bioactive compounds and proliferation of *E. coli*. Bioactive compounds are extranutritional compounds which exist in small amounts in plants. They are divided into different sub-groups and there have been many studies on their effect on human health. Ascorbic acid or vitamin C which is a bioactive compound was chosen as a model compound in this research. In the research project which was done by me to study the effect of bioactive compound on the propagation of *E. coli* in the third semester, it was concluded that ascorbic acid doesn't have an inhibitory effect on *E. coli* growth. The general objective of the research is to produce safe and healthy fruit and vegetables, consumed after minimal preparation, and to increase produce safety through

cultural management. The study was done in two steps; – 1) direct effect of AA on *E. coli* and 2) verification in a produce-based approach.

In this research it was tried to look at the problem from broader view. In order to have a holistic view, the socio-economic effects of the *E. coli* outbreak on vegetable retailers and consumers were investigated to see what socio-economic effects *E. coli* outbreak has had on them and what have been the reasons. Therefore the role of different segments of the society in the time of the outbreak was studied. It was tried to see what had been the role of media and authorities and how they can play a role in adopting a new way of controlling the bacteria in future; if there would be a possibility. Both social and lab work was done in this research to suggest a more sustainable way of controlling bacteria in the food system.

The following questions guide the overall research aim:

1) Do the bioactive compounds promote the propagation of verotoxin producing *E. coli*?

2) Do leaf lysates promote the survival of *E. coli* and may this be linked to the level of bioactive compounds?

3) What socio-economic effects have the *E. coli* outbreak had on vegetable producers, retailers and consumers?

### 1.1 History and origins of *E. coli* O157:H7

*Escherichia coli* O157:H7 was first recognized as a human pathogen after two outbreaks of gastrointestinal illness in the United States which were due to the consumption of raw hamburger patties. A potent toxin that killed African green monkey kidney cells (aka Vero cells) was produced by certain strains of diarrheagenic *E. coli*, so the cytotoxic agent came to be called verotoxin (VT). VT has been called as Shiga-like toxin (SLT) because there are some likenesses in immunological and also function of VT and the Shiga toxin produced by *Shigelladysenteriae* (Bach et al., 2006).

More than 100 stereotypes of *E. coli* are capable to produce cytotoxins. This group of organisms have been called verocytotoxin producing *E. coli* (VTEC), Shiga-like toxin-producing *E. coli* (SLTEC), and Shiga toxin-producing *E. coli* (STEC) (Bach et al., 2006).

Infection with *E. coli* O157 is of particular concern as it is capable of causing severe disease. Some strains of STEC are capable of causing hemorrhagic colitis (bloody diarrhoea) and

hemolytic uremic syndrome (HUS) in humans. This subgroup is named enterohemorrhagic *E. coli* (EHEC). In fact the major EHEC serotype which can cause foodborne disease in North America and the United Kingdom is *Escherichia coli* O157:H7 (Bach et al., 2006).

It has been proven that *E. coli* O157:H7 evolved from *E. coli* O55:H7, which is an enteropathogenic strain of *E. coli* (EPEC) long associated with diarrhoea in new-borns (Bach et al., 2006).

*Escherichia coli* O157:H7 cannot be excluded from the faeces of livestock; therefore, its presence in animal manures and slurries is unavoidable. Faeces of wild birds like starlings and gulls can transfer *E. coli* O157:H7 as well. Leafy vegetables are most commonly associated with the *E. coli* infection (FAO/WHO, 2008).

### 1.2 Socio-economic effects of foodborne outbreaks

Regarding the complexity of farming and food system, in addition to production, social and ecological aspects should be taken in to account (Breland et al., 2007).

Food safety is an issue which can disrupt markets and cause significant losses to farmers, consumers and marketers. When an outbreak happens it tends to affect all produce consumption. Even when the outbreak occurs far from where people live, consumers will refuse to consume the product for a while. In fact they will reduce their consumption of all "fresh produce" (Fan et al., 2009).

This fact that how quick producers can manage the contamination problem and persuade buyers that their product doesn't have a risk anymore is an important factor in the economic effect of foodborne illness outbreak. Human infections related to *E. coli* O157:H7 mostly occur in summer-time from May through September. Foodborne diseases (FBD) can also have a devastating impact on the economy of different countries by affecting their tourism industry since tourists do not want to return to a country where they have become sick (Bach et al., 2002).

Furthermore there has been an expansion in pathogen range. As has been mentioned in Wallace et al. (2011) about 50 years ago there were four main foodborne pathogens while today there are around 30 foodborne pathogens. There are some factors responsible for the spread of these pathogens:

-Modern transportation systems which allow the transport of large quantities of crops, animals and people

- The ability of microorganisms to adapt themselves to the changing environment

- Climate change; as the planet warms, the geographic range of pathogens expand from tropical to temperate regions

- War, poverty and famine which occurs around the world put more stress on human populations, increasing their susceptibility to infectious disease

- The lack of will at governmental and intergovernmental levels to take action in order to better protect public health.

### 1.3 Transmission of enteric pathogens in the environment

Although the exact mechanism of contamination in most produce-related outbreaks is not clear, lots of field research has attempted to explain the ecology of the growing environment. There are different sources associated with the transmission of bacteria in the field, for instance farm workers, machinery and equipment used in the farm. Pre-harvest stage is the main cause of leafy vegetables' contamination such as lettuce and spinach in many outbreaks (Islam et al., 2004).

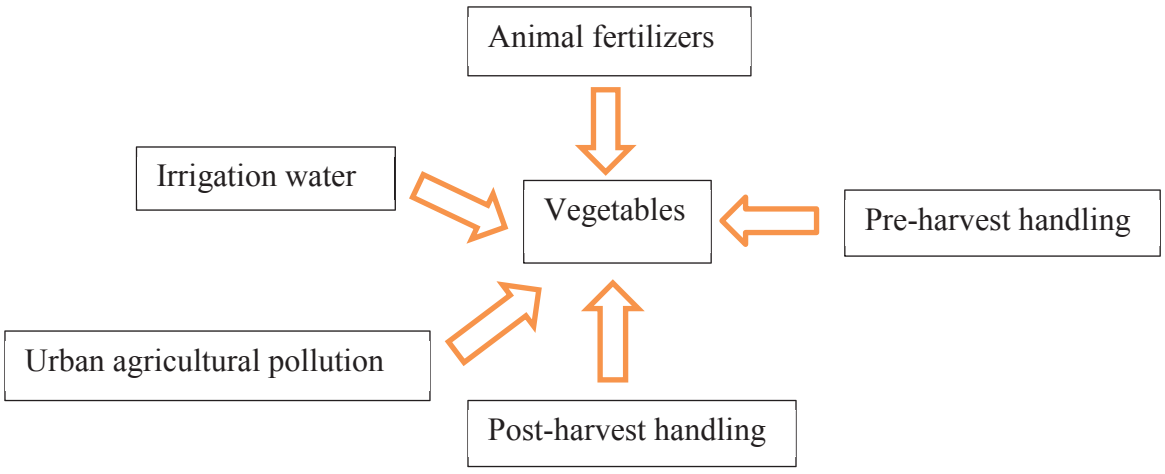


Figure 1. Potential sources of human pathogens (Fan et al. 2007)

Also irrigation water, faeces, soil amendments, organic fertilizers and wild and domestic animals are considered to be a potential source of contamination at the pre-harvest stage. Due to more intensive agriculture these days, and consequently the proximity of fields of produce to animal production zones, the ecological connections between wild animals, farm animals, and produce have become closer (Lynch et al., 2008).

Vegetables can become contaminated with pathogens at the pre-harvest stage through the use of inadequately composted manure in fields as fresh fertilizer. Both conventional and organic vegetable producers usually add animal manure to fields as fertilizer (Islam et al., 2004).

Furthermore water is always a well-recognized factor in the transmission of bacteria. For bacteria such as *Salmonella* and *E. coli* O157 it has been reported that contamination has occurred mostly through irrigation water and animal manure (Buck et al., 2003). In fact the contamination of water mainly in lettuce and spinach has been mentioned as a major cause of *E. coli* outbreaks (Ottson et al., 2011). Waste water is another important source of enteric pathogens among soil in developing countries due to its common use in agricultural irrigation (Santamaria et al. 2003).

Urban agricultural pollution is mainly related to biological and chemical pollution. Some urban agricultural practices like inappropriate crop selection regarding soil and water pollution in the area and the applying of untreated organic manure to vulnerable crops can be considered as among the health risks of urban agriculture (Armar-Klemesu., 1999).

Moreover bacterial pathogens can enter into plant tissues through different routes. Bacteria can easily enter at a scar, bruise or wound in the fruit or leaf‘s surface through irrigation water with “capillary action” at the pre-harvest stage (Fan et al., 2007).

### 1.4 *E. coli* in leafy vegetables

According to the FAO/WHO Expert Meeting “leafy vegetables and herbs, include all vegetables and herbs of a leafy nature and of which the leaf (and core) is intended to be consumed raw, e.g. lettuce (all varieties), spinach, cabbages, chicory, leafy herbs (e.g. cilantro, basil, parsley) and watercress (FAO/WHO, 2008).”

Nowadays minimally processed and ready to eat fruits and vegetables are considered an important part of a healthy diet. In fact there is an international movement to increase their

consumption and, interestingly, the consumption of vegetables by consumers has increased by 8% from 1990 to 2005 (Fan et al., 2009).

The number of outbreaks of foodborne disease related to the consumption of fresh fruits and vegetables has augmented since the early 1990s. For example, in the USA between 1998 and 2002, 2.9% (192/6647) of total foodborne outbreaks were related to vegetables. Bacteria such as *Salmonella enterica* and *E. coli* O157:H7, both previously related to illness from food of animal origin, has caused the highest proportion of epidemics associated to fresh produce that has an identified etiologic agent (Brandl, 2006).

According to Fan et al., (2009) "Leafy green consumption between 1996 and 2005 increased 9% compared to the previous decade, but outbreaks associated with leafy greens increased 38.6%, with a majority of them caused by *E. coli* O157:H7".

A reason for this is the belief that the daily consumption of fruits and vegetables will prevent certain diseases such as cardiovascular diseases and some cancers, which is true. Minimally processed or "fresh-cut" products are provided from plants' leaves which are cut to small-size pieces, washed and packed into polymeric film bags (Mackelar et al., 2011).

### 1.6 EHEC in Sweden

According to Bengtsson et al. (2010) "VTEC was only sporadically detected in Sweden until 1995 when 114 human cases of O157:H7 were notified". In 2010, 334 human cases were reported which shows an increase from 2009 (228 cases). This increase was mainly seen for the domestic cases. In 2010, 194 domestic cases were reported (59% of the total number). Children under 10 have been the ones affected in almost half (48%) of domestic cases. 34% of domestic cases involved children under 5. Some of the cases involved infections abroad. In 2010 Egypt was one of the countries in which the most cases of disease were reported (40 cases) (Bengtsson et al. 2010).

## 2. Materials and Methods

Data sources were identified in accordance with the type of research. Research tools, which in this case were standard lab tests and interviews, were utilised. Finally statistical procedures for data analysis were employed.

## 2.1 Quantitative research methods

Experimental research was employed for this research project. In this kind of research, scientific methods are followed more than any other research methods. Data used in this research includes standardized test results and interviews. In conducting an experimental research study the direct management of the independent variable and also the control of irrelevant variables are necessary (Taylor, 2005). In the current research in one step impact of ascorbic acid as a bioactive compound on the propagation of the two selected strains was investigated. In the second step the leaf lysates interaction with *E. coli* was examined. It was to investigate that the leaf lysate promote the survival of *E. coli* in the presence of bioactive compounds.

### Laboratory experiments

In this project, bacterial growth was investigated using classical microbiological methods. The experiment was done in two steps. In step 1, bacterial growth in the presence of ascorbic acid and mineral medium was investigated. In step 2, bacterial growth in the presence of leaf lysates was tested. Each of the mentioned steps of the experiment executed during 72 h.

### 2.1.1 Source of bacterial strains

Two strains of *Escherichia coli*; *E. coli* K12 (kindly provided by Dr. Jim Monaghan, Harper Adams, UK) and a biochemical strain of *E. coli* O157:H7 (registry no. E81186; kindly provided by the Swedish Institute of Communicable disease control, Stockholm) were used in this study.

### 2.1.2 Microbiological media

The following microbiological media were used for the different steps within the laboratory experiments. The incubation conditions and experimental step are provided in table 1.

Table1. Microbiological media and their incubation conditions for the different *E. coli* strains used in the present thesis. The experimental steps, in which the medium was used, are displayed.

| Microbiological medium | Incubation temperature (°C) | Incubation length | Comments | Experimental step |
|---|---|---|---|---|
| LB | 37 | 18 | | Pre-culturing of *E. coli,* |

| | | | | |
|---|---|---|---|---|
| | | | | calibration curve |
| LB broth | 37 | 18 | Used as either single or double strength | Experiment 2, Pre-culturing of *E. coli* |
| Minimal mineral medium | | | | Experiment 1 Experiment 2 |
| Physiological NaCl (0.85 %) | | | | Experiment 1, Experiment 2, Calibration curve |

1) LB

| | |
|---|---|
| LB broth (L3022, Sigma-Aldrich) | 20.0 g |
| Agar | 15.0 g |
| Distilled water | 1000.00 ml |

2) LB broth

| | |
|---|---|
| LB broth (L3022, Sigma-Aldrich) | 20.0 g |
| Distilled water | 1000.00 ml |

3) Mineral medium was prepared according to the recipe presented by DSMZ (2008) in a three-step-approach, starting with SL-6, followed by SL-4 and finally the main solution. The stock solutions, SL-4 and SL-6, were membrane filtered (0.20 μm) into sterile flasks and kept in the refrigerator at 4 °C until use. The main solution was freshly prepared for each experimental step and membrane filtered before use.

| | |
|---|---|
| $Na_2HPO_4$ x 2 $H_2O$ | 3.50 g |
| $KH_2PO_4$ | 1.00 g |
| $MgCl_2$ x 6 $H_2O$ | 0.10 g |
| Trace element solution SL-4 (see below) | 1.00 ml |
| Distilled water | 1000.00 ml |

PH 7.25

*Trace element solution SL-4*:

| | |
|---|---|
| EDTA | 0.50 g |
| $FeSO_4$ x 7 $H_2O$ | 0.20 g |
| Trace element solution SL-6 (see below) | 100.00 ml |
| Distilled water | 900.00 ml |

*Trace element solution SL-6:*

| | |
|---|---|
| $ZnSO_4$ x 7 $H_2O$ | 0.10 g |
| $MnCl_2$ x 4 $H_2O$ | 0.03 g |
| $H_3BO_3$ | 0.30 g |
| $CoCl_2$ x 6 $H_2O$ | 0.20 g |
| $CuCl_2$ x 2 $H_2O$ | 0.01 g |
| $NiCl_2$ x 6 $H_2O$ | 0.02 g |
| $Na_2MoO_4$ x 2 $H_2O$ | 0.03 g |
| Distilled water | 1000.00 ml |

4) Physiological NaCl (85%)

| | |
|---|---|
| NaCl | 8.5 g |
| Distilled water | 1000.00 ml |

Complex microbiological media and physiological NaCl were sterilized by autoclavation at 121 °C for 20 min. Solid media, containing agar, were cooled to 50 °C before pouring 20 ml in sterile disposable petri dishes. Prepared plates were stored in sterile bags upside down in the cooler (4 °C) until use. Flasks containing autoclaved liquid medium (broth) was cooled and stored at room temperature until use.

### 2.1.3 Preculturing of *E. coli*

The *E. coli* strains were kept in stock in cryo-cultures at -80 °C. For each experiment, a fresh culture was prepared from the frozen stocks by streaking out 20 µl of bacterial suspension (quadrant method) on LB and cultured at 37 °C for 18 h. For each experiment, cells from one colony were transferred to 2 ml of LB broth and cultured on a rotary shaker (200 rpm) at 37

°C for 18 h. Before inoculation, cells were washed by repeated centrifugation and resuspension in 0.85 NaCl.

### 2.1.4 Experiment 1: Interactions between *E. coli* and ascorbic acid

**Preparation of ascorbic acid:** The process was done in four steps, starting with preparing 200 mM solution, followed by 20 mM, 2 mM, 0.2 mM. 1.7613 g ascorbic acid was weighed at first and 10 ml deionized water was added to it in a beaker. The solution then was filter sterilized (pore size: 0.45 µm; Acrodisc Syringe Filters, Pall corporation, USA, South Wanger Road). one tenth dilutions was applied preparing different concentrations.

200 mM concentration

| | |
|---|---|
| Ascorbic acid (Sigma A-5960, St. Louis, MO) | 1.7613 g |
| Deionized water | 10 ml |

**Plate preparation (1):** The loading of the plates was done immediately after the preparation of different concentrations of ascorbic acid using microtiter plates. As the experiment was done using two strains of *E. coli*, 200 µl of *E. coli* K12 in the first step and *E. coli* O157:H7 in the second step were transferred into the all treatments except negative control. In order to transfer the compounds to the microtiter plates a multichannel pipette which was adjusted to 150 µl was used.

| | |
|---|---|
| Treatment 1 | 2 ml water, 2 ml mineral medium (negative control) |
| Treatment 2 | 2 ml ascorbic acid (100 mM), 2 ml mineral medium, bacteria |
| Treatment 3 | 2 ml ascorbic acid (10 mM), 2 ml mineral medium, bacteria |
| Treatment 4 | 2 ml ascorbic acid (1 mM), 2 ml mineral medium, bacteria |
| Treatment 5 | 2 ml ascorbic acid (0.1 mM), 2 ml mineral medium, bacteria |
| Treatment 6 | bacteria, 2 ml water, 2 ml mineral medium (positive control) |

As soon as all microtiterplates (5 replicates) were filled, they were incubated in the Omnilog (Biolog Corporation Hayward CA.USA). Growth was monitored in the Omnilog reading the plates every 15 minutes for 48 hours at 37 °C.

### 2.1.5 Experiment 2: Interaction of *E. coli* O157:H7 in leaf lysates

**Leaf lysate extraction:** red Swiss chard (batch number: 7330583001709), Rocket (batch number: 510113566-3) and Mizuna (batch number: 7330583003888) were used as model crops. Middle leaves were selected to produce lysates. At first 100 g of leaves were put in the smasher (AES Laboratore, Chemunex, France, Rue MryesBastil) (3 min, strong mode), after rapidly crunching the leaves with a sterile pestle in a sterile ceramic mortar. The obtained lysates from each crop were then centrifuged at 12000 g for 6 minutes (4 °C), immediately filtered (pore size: 8 µm; Acrodisc Syringe Filters, Pall corporation, USA, South Wanger Road). The supernatant was filter sterilized (pore size: 0.45 µm; Acrodisc Syringe Filters, Pall corporation, USA, South Wanger Road) in the sterile hood and the filtrate was used for the inoculation experiment.

**Plate preparation (2):** This step was done immediately afterleaf lysates extraction. Fresh cells of a gfp-mutant of *E. coli* O157:H7 was used in the experiment (100µl).10 mM ascorbic acid was used in this step applying one tenth dilution. Transferring the compound to microtiter plates was done with a multichannel pipette adjusted to 130 µl.

Treatment 1 2 ml LB, 2 ml ascorbic acid, bacteria

Treatment 2 2 ml LB, bacteria (negative control)

Treatment 3 2 ml Rocket lysate, bacteria

Treatment 4 2 ml Mizuna lysate, bacteria

Treatment 5 2 ml red Swiss chard lysate, bacteria

Treatment 6 2 ml mineral medium, 2 ml ascorbic acid, bacteria

After filling up all microtitre plates (5 replicates), they were placed in the Omnilog (Biolog Corporation Hayward CA.USA) at 37 °C for 96 h to be incubated.

**Determination of Ascorbic Acid:** A total of 5 g of leaves of Mizuna, Rocket and red Swiss chard, used for lysate extraction, were chopped with a knife and placed in 50 mL plastic tubes. Aliquots of 20 ml of meta-phosphoric acid were added to the tubes and they were homogenized with a laboratory mixer for 1 minute. Samples were stored in the freezer at -80 °C for two days.

Immediately before analysis, the samples were thawed in luke warm tap water. The leaf extracts were then transferred into 1.5 ml Eppendorf-tubes. HPLC analysis was performed after centrifuging the samples for 10 minutes at 13000 rpm at 4 °C. Then 500 µl was added into autosampler/HPLC vials for HPLC analysis. By subtracting the ascorbic acid concentration from the total vitamin C concentration which was obtained after a reduction procedure, the dehydro ascorbic acid concentration was calculated. Then it was used for high performance liquid chromatography (HPLC) analysis. Aliquots of 500 µl of the extract were added to a 1% dithiothreitol (DTT) solution and the pH was adjusted with $K_2HPO_4$. Thereafter the ascorbic acid was measured through HPLC analysis. Solvent of HPLC analysis was 1.5% Metaphosphoric acid, and retention time 4 minutes; a full run is 15 minutes. Detection was DAD, diode array detector, at 248 nm wave length.

**2.1.6 Calibration curve for density measurement of bacterial inoculum:** Bacteria were precultured for 18 h at 37 °C. The prepared cells in liquid culture were centrifuged (Beckman Coulter, Avanti J-20, USA) at 12000 for 6 minutes (4°C). The OD 1, 0.5, 0.4, 0.35, 0.3, and 0.2 was determined using 200 µl NaCl using a microplate reader (AsysHitech GmbH, Astria). To reach OD 1, 1.7 ml of bacterial suspension was added to 0.3 ml NaCl. For 0.5, 0.9 ml bacteria were added to 1.1 ml NaCl. To reach OD 0.4, 0.7 ml bacteria were added to 1.3 ml NaCl. To reach 0.35, 0.5 ml bacteria were added to 1.5 ml NaCl. To make OD 0.3, 0.4 ml bacteria were added to 1.6 ml NaCl. Finally to reach OD 0.2, 0.2 ml bacteria were added to 1.8 ml NaCl.

The dilution series from each sample was made; for OD 1, -9 and -10 was applied. For other ODs respectively up to -6, -4 and -3 dilution were applied. 3 plates from each selected dilution were made using WASP 50ul (Don Whitley Scientific Limited, 2001). The plates were placed in the incubator for 18 hours at 37 °C. The next morning the colonies were counted using a counting grid according to the WASP user manual.

A calibration curve was then drawn for the different concentrations of bacteria inoculum. The optical density of the bacteria solution which was applied in the experiment was 0.35 which according to figure 5 represents 8.5006 $logcfuml^{-1}$.

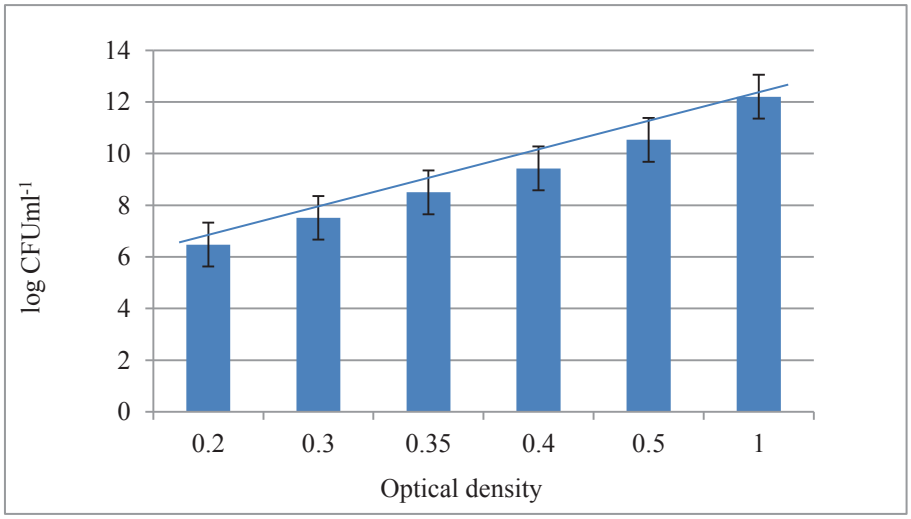


Figure 5. Dilution fact regarding different optical density was measured using WASP. Five dilutions were applied in the experiment; 0.2, 0.3, 0.35, 0.4, 0.5, 1 (0.2= 6.477121255, 0.3= 7.514104821, 0.35= 8.500602351, 0.4= 9.425968732, 0.5= 10.53571597, 1= 12.20411998)

### 2.1.7 OmniLog:

The OmniLog detects increasing darkness in wells as color is formed. It functions as a densitometer, measuring the decreased light reaching the pixels of the camera that correspond with each individual well. Plates are back-lit with diffuse white light. On each read, a plate image is taken and the light intensity (I) for each of the 96 microplate wells is calculated compared to full light intensity (I0 = 100% transmittance) measured over the same pixel area. Beer's law allows the calculation of an OD value according to the equation:

OD = - log10 (I/I0) so 100% transmission = 0.0 OD, 10% = 1.0 OD, 1% = 2.0 OD

An OL unit is calculated as 500 times the OD, with a maximum value of 400. The actual linear range of the OmniLog is up to approximately 225 OL units.

The following tables show approximate relationships between transmission, optical density and Omnilog units (table 1 and 2) (Bochner, personal communication to B. Alsanius).

Table 1: Approximate relationships between transmission, optical density and omnilog units (according to Bochner, 2011; personal communication to B. Alsanius)

| Transmission | Optical density | Omnilog units |
|---|---|---|
| 1 | 0.000 | 0.000 |

| | | |
|---|---|---|
| 0.9 | 0.046 | 22.879 |
| 0.8 | 0.097 | 48.455 |
| 0.7 | 0.155 | 77.451 |
| 0.6 | 0.222 | 110.924 |
| 0.5 | 0.301 | 150.515 |
| 0.4 | 0.398 | 198.970 |
| 0.3 | 0.523 | 261.439 |
| 0.2 | 0.699 | 349.485 |
| 0.1 | 1.000 | 500.000 |

Table 2: Approximate relationships between transmission, optical density and omnilog units (according to Bochner, 2011; personal communication to B. Alsanius)

| Omnilog units | Optical density | Transmission |
|---|---|---|
| 400 | 0.8 | 0.158 |
| 350 | 0.7 | 0.200 |
| 300 | 0.6 | 0.251 |
| 250 | 0.5 | 0.316 |
| 200 | 0.4 | 0.398 |
| 150 | 0.3 | 0.501 |
| 100 | 0.2 | 0.631 |
| 50 | 0.1 | 0.794 |

## 2.2 Qualitative research methods

As a complementary research the socio-economic effects of *E. coli* outbreak on vegetable retailers and consumers were investigated. Qualitative research methods were applied in order to organize open-ended interviews in the research. According to Taylor (2005),"qualitative research is multi-method in focus, involving an interpretive, naturalistic approach to its subject matter." The aim was to analyze people's experiences, perceptions and opinions about the research subject through interviews.

### 2.2.1 Interview and survey

The interviews were conducted in order to understand to what extent people, industry and growers have been affected with *E. coli* outbreak and why. The purpose was to recognize and to reveal the extent of people's awareness about the *E. coli* outbreak and to measure the consequences of it on their food habits. The interviews also aimed to explore the role of different segments of society such as government, media, and education at the time of the outbreak. In carrying out the interviews an interview guide was used.

Semi-structured interviews of quality control managers at ICA, SABA and Grönsaksmästare were conducted as a complementary method in this research. A survey was conducted with the consumers. 50 consumers were selected randomly to be interviewed in "Willys" and 50 in "Coop".

Grönsaksmästare: "FridaStenarsson", quality manager of Grönsaksmästare, was interviewed in this company.

ICA: ICA is one of the leading retail companies throughout Sweden and in the Nordic region and is a hundred years old. A food quality manager of fruits and vegetables in ICA, "Anders Axelsson", was interviewed.

SABA: SABA is one of the main importers and distributors of fruits, vegetables and flowers in Northern Europe. "Bengt Petersen", a quality manager of "SFC" (SABA fresh cuts) Company, was interviewed.

Afterwards two questionnaires were prepared, one to cover quality managers and one to cover consumers. A written list of specific topics and questions to be covered in a particular order was prepared.

In the consumers' survey, respondents were asked the same questions in a precise manner and were each offered the same set of possible responses. This style of interviewing avoids starting a conversation with the respondents. Sometimes when the questions are not clear enough respondents will need to get clarifications from the interviewer (Bernard, 2006).

"Personal face to face interviews" were used in this research. The advantage of face-to-face interviews is that the interviewer and respondents can experience a connection. Therefore the interview process can be carried out more easily when the interviewer can see who he or she is interviewing. But one of the disadvantages of "face to face interviews" is that they are costly in terms of both time and money (Bernard, 2006).

In addition in a face-to-face interview, if a respondent doesn't understand a question, the interviewer can explain it or, if the interviewee does not answer fully, they can be "probed" for more information. According to GAO (1991), "In comparison with mail questionnaires, face-to-face and telephone interviews are much faster methods of gathering data."

When conducting interviews the "probing method" has been mentioned by Bernard, (2006) as a key for achieving success. It means encouraging the respondents to give more information without leading them to a specific answer which the interviewer desires. ''The silent probe'' was applied as one "probing method" when interviewing the food quality managers. The "echo probe", which simply involves repeating what the respondents say and asking them to continue, was also used in some interviews. But mostly in this research the "Tell-me-more" and "Uh-huh" probe was used to encourage the respondents through an affirmative comment (Bernard, 2006).

### 2.2.2 Question format

In designing an interview many considerations should be taken into account; the specific questions, the question format, layout, and language order. There are different kinds of question format. When interviewing the consumers most of questions were in the form of "fill in the blank" questions. Also "Binary-Choice Questions", which means "yes-no" or "true-false" questions were applied in the inquiry as well (GAO, 1991).

When interviewing the food quality managers, open-ended questions were applied. The fact is that in open-ended methods the interviewer is fully in control of what he or she wants from an interview and is able to follow new leads (Bernard, 2006).

Since “closed questions” seemed more efficient and also clearer for the purposes of analysis most of the questions to the consumers in this research were designed using such a format.

The most important thing when conducting an interview is to avoid asking ambiguous questions. Also the vocabulary used in a survey is important. The appropriate wording of questions is helpful in interviews. In order to conduct a successful interview the language level of the interviewer and the respondent should be the same and the respondents should feel comfortable with the level of language used by the interviewer.

Furthermore respondents should know enough about the survey’s subject to be able to respond. In addition any question should appear to have a clear purpose for both the interviewer and the respondents (Bernard, 2006).

In question formatting I kept making short questions. I avoided asking “loaded” questions; which means any question which starts with a leading concept. For instance “Don’t you agree that…” is a “loaded” question.

### 2.2.3 Ethical issues

Ethical issues can sometimes pose difficulties when conducting research. Bernard (2006) has concluded ‘’what’s popularly ethical today may become popularly unethical tomorrow.’’ There are some aspects which should be taken into account when conducting an interview such as “informed consent”, “confidentiality” and the “consequences” of the interview to the interviewees. Also the interviewees should be aware of the purpose of the interview. In the semi-structured interviews before recording the interviews respondents were asked if they agreed and consented to it. The validity and reliability of the gathered data was clarified as well. In order to do so an attempt was made to “keep bias out of the data by reporting only what was observed and told rather than inferring what was believed to have been told.” Also participants had a chance to review the gathered information for accuracy.

## 2.3 Data analysis

The data was analysed using the programs Excel and Minitab 16. The average of the different wells was formed and these averages were related to the corresponding viable count. Anova one-way analysis was performed with Minitab. The differences between the two means were determined through Tukey’s multiple comparison tests ($p<0.05$).

Data regarding consumers' survey was analysed using the programs SPSS and Excel. To measure the association between different variables, multi-regression analysis was carried out.

In order to analyse the semi-structured interviews, the tape recordings were transcribed to text. During this process the "main themes" in the recordings were written down as I listened to the tapes. When analysing the text, the main ideas of each question or topic were identified. The "meaning condensation method" was applied for analysing semi-structured interviews. This method is based on finding the meaning units of the text and then paraphrasing it.

# 3. Results

## 3.1 Laboratory experiments

### 3.1.1 Bacterial propagation in the presence of ascorbic acid

In phase one; bacterial propagation (2A= *E. coli* k12, 2B= *E. coli* O157:H7) in the presence of different concentrations of ascorbic acid and mineral medium in 48 h of incubation in the omnilog was tested. There is a growth only in 10 mM concentration of ascorbic acid (figure 2 A, B). Interestingly in other concentrations of ascorbic acid (0.1, 1, 10, 100) no growth was observed.

All stages of bacterial growth are observable in figure 2A. Bacterial growth in the "log phase" is rapid. Bacteria enter the "stationary phase" in the next step and then go into the decline phase before descending gently until the end of incubation. The growth in the 10 mM concentration of figure 2B is slower than in figure 2A in the whole process. But bacteria cultured in a suspension supplemented with 10 mM of ascorbic acid never reached the stationary phase within the 48 h of incubation. As in figure 2A, no growth was observed of the other treatments (figure 2B). Grouping information using Tukey's method showed the difference of means in letters. Means which do not share a letter are significantly different. In figure 2, 10 mM concentration is represented by letter A. Other treatments are shown by letter B.

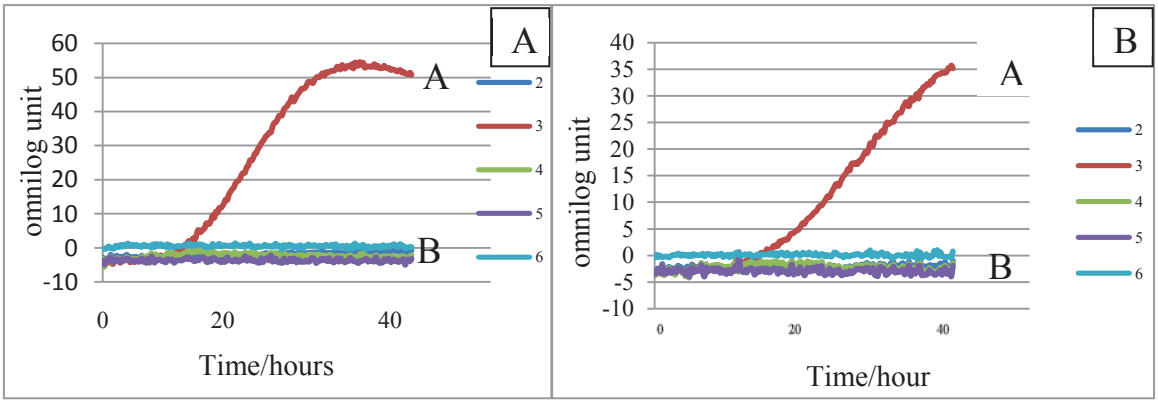


Figure 2. Bacterial propagation (model strain: *E. coli* K12, A and *E. coli* O157:H7, B) in different concentration of ascorbic acid in the presence of mineral medium during 48 h of incubation using the Omnilog system (2= 100 mM, 3= 10 mM, 4= 1 mM, 5= 0.1 mM, 6= positive control). All values were zero-adjusted on the basis of the non-inoculated control (negative control; treatment 1). (n=5)

### 3.1.2 Bacteria propagation in the presence of leaf lysate

As mentioned three crops were used in step 2 of the experiment: rocket, mizuna and red Swiss chard. As the reddish color of lysates from red Swiss chard interfered with the absorbance spectrum of the spectrophotometer, propagation of the inoculated strain could not be monitored using this method and was therefore excluded.

Treatment 1 in figure 3 is positive control. It represents *E. coli* O157:H7 growth in the presence of ascorbic acid and LB broth. During log phase a considerable increase of microbial cells was found, as scrutinized by repeated Omnilog readings. In treatment 1 the "Log phase" took 50 hours and growth was gradual.

Treatment 2, which was the negative control, examined the interaction of *E. coli* O157:H7 in the presence of LB broth. According to this figure, the "lag phase" was very short. The "log phase" started in half an hour and the growth increased gradually for 56 h to reach 70omnilog value. Bacterial growth then entered the "stationary phase" and remained stable until the end of the experiment. No decline in cell density was observed. Figure 3 and 4 characterize the interaction of leaf lysates (3= mizuna, 4=rocket) with *E. coli* O157:H7. In these two treatments, the lag phase was barely noticeable. In these two treatments, cell densities increased rapidly during log phase; and reached after 6 h to 100 omnilog value. Stationary phase started after 60 h. Interestingly no decline in optical densities was observed in the later phase when *E. coli* O157:H7 was incubated in lysates of rocket and mizuna. After 96 h of incubation, rocket and mizuna reached almost 163 and 160omnilog value, respectively.

Growth pattern in these two different crops were similar. But the general growth in leaf lysates of rocket were higher than in the ones obtained from mizuna.

Finally figure 6 shows the interaction of *E. coli* O157:H7 with mineral medium and ascorbic acid. In this treatment the lag phase was much more extended as compared to all other treatments (around 4h). The log-phase was slow and it took around 48 h of incubation to reach maximum optical densities of 58. The stationary phase was very short and decline in optical density was observed quickly after. Treatment 3 and 4 in which the means are close to each other are presented by character "A" in Tukey's method. Treatment 1 which is represented with character "BC" in the first 18 hours and "B" for the remaining hours, share letter "B" with treatment 2 for almost the whole process of growth. Treatment 6 is characterized with the letter "C".

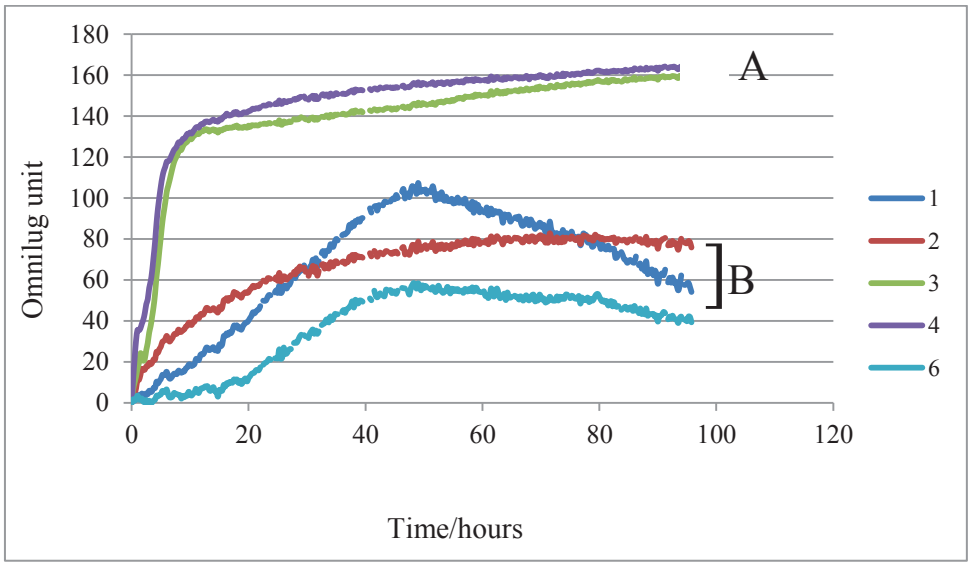


Figure 3. Interaction of leaf lysates on survival and propagation of *E. coli* O157:H7 in presence of bioactive compounds for 96h in Omnilog (1= LB+ ascorbic acid+ *E. coli*, 2= LB+ *E. coli*, 3= Mizuna + *E. coli*, 4= Rocket+ *E. coli*, 6= mineral medium+ ascorbic acid+ *E. coli*).

### 3.1.3 Ascorbic acid measurement

Determination of ascorbic acid content in leaves from the three different crops (red Swiss chard, mizuna, rocket) showed (figure 4) that the content was highest in rocket (almost 500 mg AA (kg fw)$^{-1}$, whereas mizuna and red Swiss chard contained almost 340 mg AA (kg fw)$^{-1}$ and 200 mg AA (kg fw)$^{-1}$.

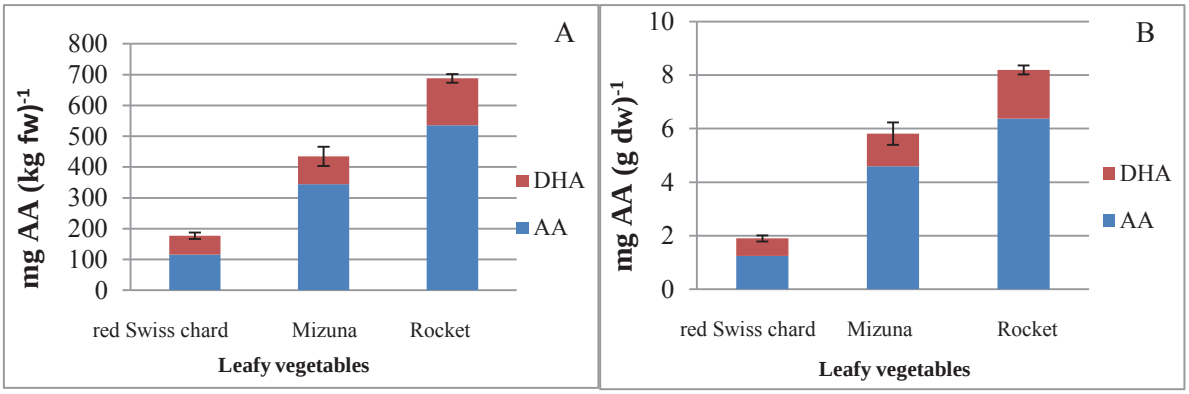


Figure 4. Ascorbic acid content in fresh (A) and dry (B) matter of red Swiss chard, mizuna and rocket. n=3. AA= ascorbic acid, DHA= dehydro ascorbic acid. fw= fresh weight, dw= dry weight.

## 3.2 Interview result

### 3.2.1 Consumers' survey

100 consumers were interviewed in "Coop" and "Willys" on different days. Interviews were conducted on Saturdays as the aim was to interview people from different age groups. Also interviews were conducted at different times of the day; in the morning, afternoon and evening.

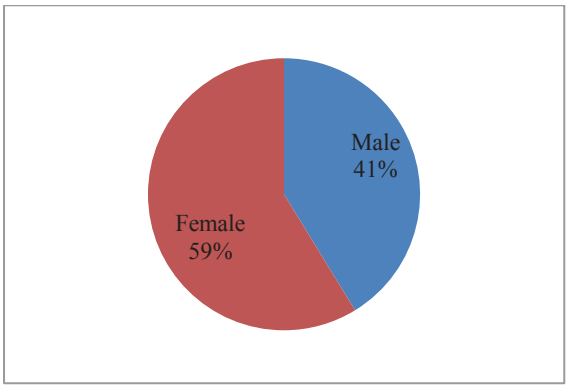


Figure 6. Gender of the respondents.

According to figure 6, 41% of respondents were male and 59% were female.

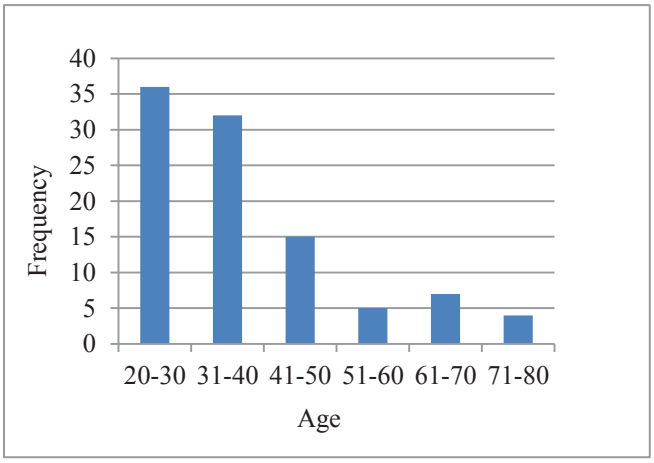


Figure 7. Participants grouped by age. There are six age groups; 20-30, 31-40, 41-50, 51-60, 61-70, 71-80 years.

In figure 7, the respondents' distribution according to different age groups is shown. Most of the respondents belonged to the age groups 20-30 and 31-40, as they were more willing to participate. The age group 71-80 has the lowest participation level.

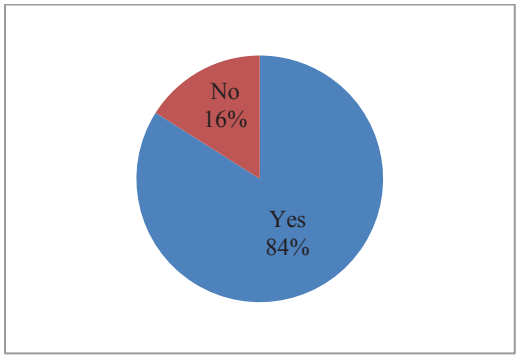


Figure 8. Respondents' awareness about EHEC outbreak; people were asked if they have heard of EHEC outbreak.

Figure 8 illustrates the awareness of people about the EHEC outbreak which occurred in the summer of 2011. Interestingly according to figure 7, 84% of the respondents were aware of the problem. Only 16% of participants had not heard of the EHEC outbreak.

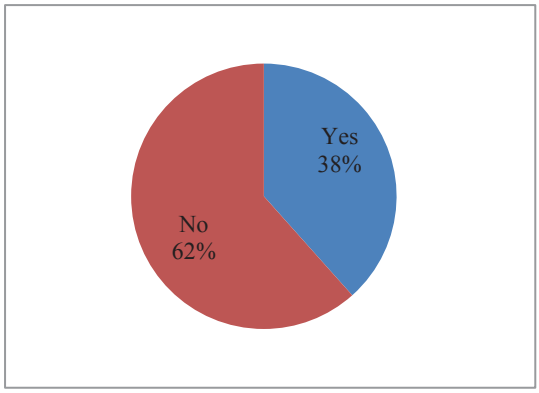


Figure 9. Respondents were asked if they knew anyone who became sick of EHEC at the time of the outbreak.

This figure shows that 38% of respondents knew someone who has become sick of EHEC and 62% didn't know. It can be according to respondent's mixing of *E. coli* with EHEC, as the number of sick people has been a few.

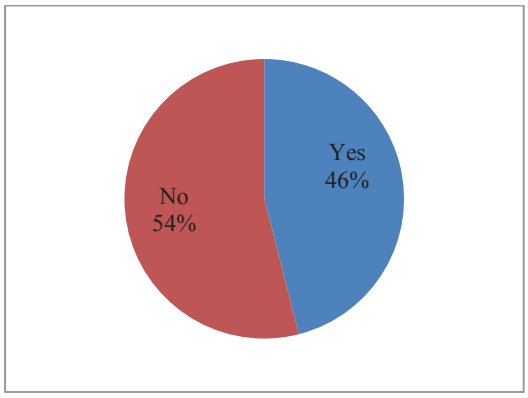


Figure 10. Peoples' awareness about the health consequences caused by EHEC-infections.

When asked about their knowledge on health consequences caused by EHEC infections, 46% of people responded that they have heard about the consequences of getting infected with EHEC. Interestingly, 54% of the respondents claimed that they do not know about the consequences.

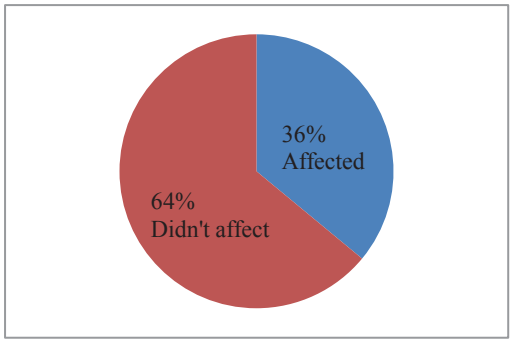


Figure 11. Impact of food habits provoked by the EHEC outbreak in 2011, expressed as 36% of respondents.

Surprisingly 64% of the respondents claimed that the EHEC outbreak in 2011 had not affected their food habits whereas the remaining 36% had altered their food habits as a result of the news. However, the impact on food habits did not last for a long time and after one month most of the respondents had returned to their usual diet (figure 12).

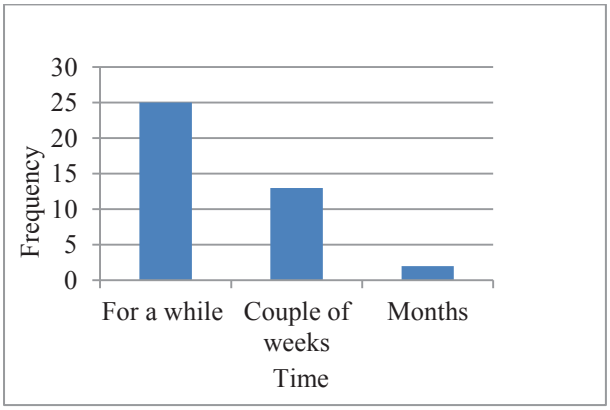


Figure 12. Duration of the EHEC outbreak's effect on peoples' food habits (the choices were for a while, a couple of weeks, or for a month)

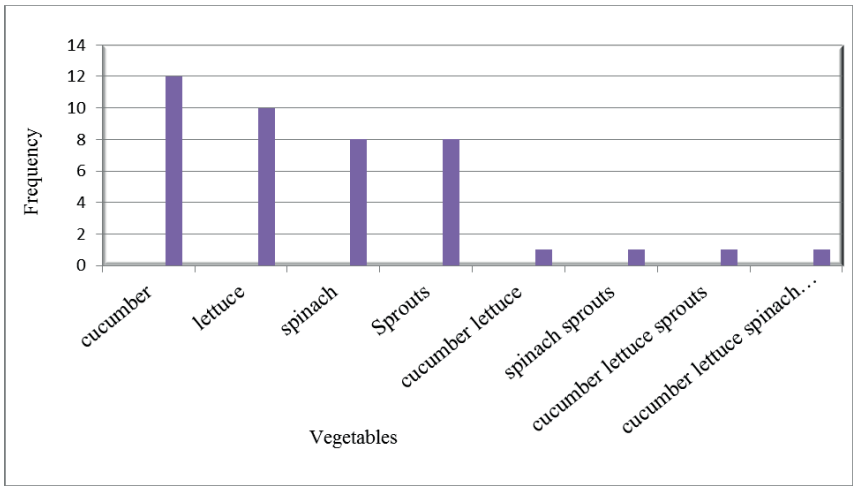


Figure 13. Vegetables which were avoided by the respondents after the *E. coli* outbreak; cucumber, lettuce, spinach, sprouts were among the choices. Other options in the graph have been chosen by people who haven't consumed two or more vegetables due to the outbreak.

The change in consumers' vegetable consumption is shown in figure 13. Obviously cucumber has been consumed less than the other vegetables following the outbreak by the respondents. Lettuce came second in terms of the products whose consumption has been affected most by the outbreak. Spinach and sprouts are the other vegetables which have been consumed less after the outbreak. Interestingly the majority of respondents avoided one main produce, only some of the respondents have avoided consuming more than one vegetable.

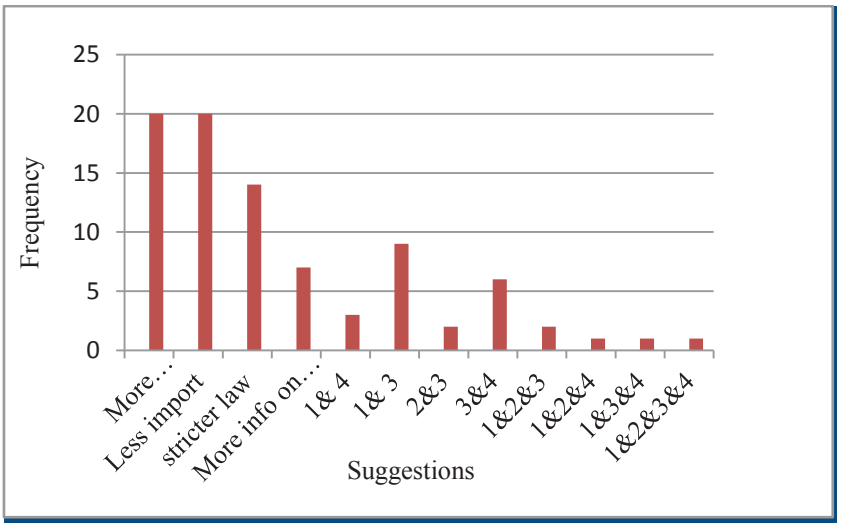


Figure 14. Respondents' suggestions for improving the situation in future (1=more monitoring of the food system, 2=less import, 3=stricter laws, 4=more information in the media).

Figure 14 displays respondents' suggestions as to how to improve the situation in the future. From the respondents' point of view more monitoring of the food system and less imports from other countries are the main measures which can address the situation. Others such as stricter laws and more information in the media have also been mentioned by consumers.

Also a multi-regression analysis was carried out to measure the association between different variables. Since all the variables were categorical statistical tests, Pearson Chi-Square and Gamma was applied. According to these tests there is no association between the questioned variables including hearing of EHEC, having knowledge about the consequences caused by infections with EHEC, knowing someone who has become sick from it, the demand for consuming vegetables, the time period and the type of vegetables that people stopped consuming due to EHEC. The only significant association found was a strong association between having knowledge about the consequences of EHEC and knowing someone who has become sick. The Gamma test value of 0.0707 ($P < 0.01$) tells us that knowing anyone who has become sick increases by 70.7% the chance of having knowledge about the consequences of EHEC.

This test neither reflected any association between gender and the other questions. But age group can affect hearing about EHEC with the Gamma test showing a strong positive relationship between the two. This means that the higher age group the greater the chance of someone hearing of EHEC is. The Gamma's value was 0.814 ($P < 0.01$) which means knowing a person's age group would improve our estimate of hearing of EHEC by 81.4%.

### 3.2.2 Interview with the food quality managers

Three in-depth-interviews were conducted with quality control managers of three different companies in order to collect information regarding the *E. coli* outbreak in 2011. According to Julie Laforest, (2009) "Under optimal conditions, data collection from key informants should end once data saturation is achieved, i.e. when interviews do not provide any new or additional insights because the information gathered is repetitive." After this "data saturation" was achieved following the third interview, data collection was stopped.

**The economic effect of *E. coli* outbreak on the companies:** The interviewees claimed that their companies have been affected economically by the *E. coli* outbreak. The effect of the outbreak on the ICA and SABA Company lasted for a short time. There was a drop in the sale of the products generally so the whole market was affected due to this problem. The

consumers stopped buying the products at that time and it seems that they are still concerned about Spanish products such as cucumber. But Grönsaksmästare has been affected more severely with respect to sprouts and shoots. "Frida" said:

> "Regarding the *E. coli* outbreak the company lost a lot which was mostly due to the low price of specific products like cucumber, tomato and sprouts. Sprouts are really small articles in the stores or on the market. If you buy sprouts four times a year and you change it to only three times a year, it will affect us a lot even if the consumers don't think about it. For a couple of weeks there was not a single box sold. We couldn't sell… for a couple of weeks. We still haven't come up to the same amount of sales; I think we are up to 80%."

**Role of the media in the time of outbreak:** Regarding the media's role the interviewees had different opinions. "Anders" said that there is a policy within ICA relating to information; they are supposed to be open and informative. In fact, ICA does not collaborate directly with the media. But they are open in answering questions and are informative whenever there is a problem.

Frida criticized the media for the role it played at the time of the outbreak. She believes that the issue was somehow misrepresented by the media. Every day there was news in the papers about people who were dying of *E. coli*. But when there is no problem anymore they do not report that fact in the news. She said:

> "In this specific case, the media was …they were rather good in the beginning. They gave the right information to people about the *E. coli* outbreak in Germany. But after a couple of weeks when we had Swedish people dying as well …there were a bit of confusing articles in the media. Like, now you can see how EHEC is spreading through Sweden…but it was just people who were living in Sweden coming from Germany, going home and being sick there. The media is usually interested in disasters ….When you did nothing wrong you want that to be on the first page in the media. When you are mistreated in some way you want to show everyone that these sprouts and shoots are really good things to be eaten."

**Role of Education:** Regarding consumers' education, none of the companies have a regular education program for consumers or suppliers. Consumers are only provided with information through labels or packaging materials. Frida believes that:

> "Food safety education for consumers should be carried out in schools… I don't know …or in national campaigns… if that works. But when it comes to us to educate the consumers we could do it by like having good information on our labels or on our packaging material."

**The effect of Authorities:** Interviewees had different ideas about the authorities' role during the outbreak period. According to Anders:

> "The government was a little bit hesitant also to react, because they knew that it wasn't a Swedish problem. They came out with some general requirements that you should avoid sprouts and beans and …. We were just keeping close contact with the authorities and if they had any recommendation we would follow them. So we didn't sell for a month or two but it's not a big product for us."

Frida on the other hand criticized the authorities for not being well informed about suppliers' and producers' concerns. She mentioned:

> "Authorities …they're highly respected in Sweden…what they say, people tend to think it's true. They have a critical role in how they make their decisions...em...em and how to tell Swedish people what to do or what not to do. In this specific case they did something that wasn't so good for Swedish growers. They…said that they didn't recommend anyone to eat sprouts.

The fact is that if the authority, instead of saying it is a recommendation not to eat certain products, had said that people weren't allowed to eat them; the growers could have received money from, for example, their insurance company. But in the prevailing situation, neither the European Union, nor the Swedish State nor their insurance companies compensate for costs due to sales decline.

**Risk assessment and management:** "Anders" said:

> "Because people have to consume something and get sick first, there is an incubation of couple of weeks or ten days. Then they have to report that they are sick and all people don't do it… so maybe someone in the health authority is beginning to put together…okay a lot of people are getting sick of something. So that might be a month from the actual outbreak at least. Then there is a problem in putting things together, "is this a big outbreak?", what is the common factor… so information, I would say is the biggest parcel. But to prevent an outbreak you have to have some form of risk assessment which puts the right demand on your suppliers."

Frida believed that:

> "In this outbreak, it was almost impossible to calculate any risk because we didn't know it existed….but what we did …was that we wanted to be sure that we don't have problem."

In addition the SABA has a program for all the suppliers in which they should take samples regarding *E. coli* and *Salmonella* every month. They send out the samples to the laboratory to be sure about a product's safety. Moreover the suppliers which are contracted to SABA should use ground water in their products.

**Consumer's responsibility:** The responsibility of consumers in terms of food safety was also discussed with the interviewees. Frida said:

> "It's good to educate the consumers, so that they keep their food in a good way, when they store it for example. And they won't do crazy stuff you know…like chopping raw chicken and salad on the same board for example. A lot of people know it of course, but for some this is breaking news."

Interviewees believed that good agricultural practices which consist of, for example, good training of employees, transportation and storage maintenance programs or microbiological sampling are fundamental for safety in food system. Frida said:

> "You can have quite a lot of information about GAPs if you are a grower and you are certified through standards like "ecosil" or "global gap" I think. You get a lot of information about what you should do. It's quite easy to get it. "

Anders said:

> "That's the basis of everything. That has to be in place. It's not the same if you have problems in Sweden or Italy. It's not the same for everything but you have to recognize what is important for you."

There has not been any published guideline relating to the *E. coli* outbreak by the companies. They mentioned that they do not have to publish, they just speak and act.

## 4. Discussion

Many things such as economic, environmental and social factors have put a lot of pressure on the global food system and so there is an urgent need for an intensive food safety management. As the world is changing, there should be new ways of thinking in food safety management because what has been done in the past may not be applicable in the future.

As mentioned above the socio-economic costs of "FBDs" is high in most countries. But if this cost was more evident, or at least assessed, governments might be forced to take immediate actions to minimise the effects. Consequently, the effect of the FBDs can be considered by national and international authorities and organizations as a first step in addressing food safety and quality problems. Consumer protection organizations and cooperatives can play a significant role in the improvement of "food quality and safety control systems" (Molins, 2007).

For the food safety assurance of fruits and vegetables from "seed to plate", all segments of society need to increase and improve their attention and reaction to food safety risks. There is

a need for growers, grower organizations, retailers and also governments to make an effort to solve the problems (Molins, 2007).

In the current experiment the interaction of ascorbic acid as a bioactive compound with *E. coli* in two environments has been verified. The importance of pre-harvest to post-harvest methods which affect the contamination, as well as the factors that enable *E. coli* O157 to enter into and persist in environments such as leafy vegetables, is still unclear.

According to figure 2 no growth is observable except in 10 mM concentration of ascorbic acid. It can be concluded that in 0.1 and 1 mM ascorbic acid, bacteria doesn't grow according to low carbon source. Also in 100 mM ascorbic acid, no growth might be as a result of inhibitory effect of pH. On the other hand it should be mentioned that in the previous project related to *E. coli* pH was measured and it didn't show any significant difference in pH of different concentrations of ascorbic acid.

According to figure 4, rocket has higher ascorbic acid content than mizuna. Interestingly according to figure 3 bacterial growth has been higher in rocket as well. But it is not clear that if this is linked to the level of ascorbic acid, as the ascorbic acid content has not been analysed in the lysates before and after the bacteria were grown. From the present study it is not obvious that which compounds were used by the bacteria and what have been the interactivities. Also as there has not been study regarding the interaction of bioactive compound with *E. coli*, therefore in order to answer these questions, the study on the nutrient utilisation of bacteria in the leaf lysate is suggested. Also it would be helpful to analyse the ascorbic acid content of leaf lysate before and after the bacteria growth in it. Furthermore study of the interaction between other bioactive compounds and EHEC or other strains of verotoxin producing *E. coli* is suggested.

As mentioned above irrigation is an important factor in crop production in most regions. The quality of irrigation water is different in various regions; therefore one suggestion would be to study if regulating the irrigation before harvest (drought stress) would affect the survival of bacteria on leafy vegetables. Also studying the effect of drought stress on the formation of bioactive compounds in leafy vegetables is of great interest.

It has been reported in a study by Kyle et al. (2010) that *E. coli* O157 has a faster growth on damaged lettuce leaves than on undamaged leaves. As the damage of the leaves is greater the bacterial growth is greater. Generally the research has revealed that behaviour of pathogens

and their interactions in plant phylosfere is dependent on the ecology of plants and pathogens. Additional studies are needed in this area in order to clear the effect of microphlora on enteric pathogen survival (Critzer and Doyle, 2010).

According to the results, when *E. coli* enters into the crop it will grow in the leaf lysates. Therefore washing the vegetables with water before using them in the kitchen will not reduce the contamination. There should be other methods of confronting the problem before it comes into the hand of customers. Thus, applying methods in the pre-harvest and post-harvest stage to reduce risk is suggested.

At condition that strong evidence on interactivities between ascorbic acid content and survival as well as proliferation of *E. coli* can be provided, several pre-harvest management factors should be reconsidered regarding ascorbic acid content of crops, i.e. temperature regime (Lee et al., (2000), Morgen et al. (2011), Kader et al. (2004)), nutrient supply (Kader et al.(2004), Hornick (1992), Lisiewska et al. (1996), Boskovic-Rakocevic et al. (2012),Ojetayo et al. (2011), Fryman et al. (1991), Agar et al. (1997)), irrigation management (Lee et al., (2000), Walid et al.(2012), Nunes et al. (1998)).

In case that the interaction between bioactive compounds and EHEC is verified, applying different cultural management strategies like alternative crop patterns or modified pre-harvest and post-harvest methods which can affect the ascorbic acid content of crops in the regions with questionable sanitary irrigation water could be addressed.

Suggesting a new and alternative method like different cultural management in risky areas to producers and retailers is not easy. As any new socio-technical change there would be resistance in the beginning. The development and use of new knowledge in agricultural production system is a complex process, ‘’it involves situational biophysical interactions, social relations and the values and perceptions of the farmer and farming family’’ (Eksvard, 2009).

A set of connected changes which reinforce each other but take place in several different areas, such as technology, the economy, institutions, culture, ecology and belief systems can be described as a transition. This gradual change requires long-term thinking with a focus on multi aspect thinking and placing a focus on learning. So a gradual, continuous process of structural change within different aspects of society is needed in food safety issues in order to generally improve the situation (Rotmans et al., 2001).

Furthermore close relationship between social and natural science, between scientists, policy makers and other stakeholders is required in a form of adaptive management in order to manage food safety issues in a changing world. The process of adaptive management is based on continuous feedback and learning. Food safety management needs to be adaptive and flexible to be able to adapt with the changing environment (Salafsky et al., 2001). Additionally having feedback from the environment and societies is important in shaping policy and actions.

Farmers and the retailer's organizations should be convinced that the new cultural management is helpful, practical and adaptable. Later on consumers should be persuaded that what kind of vegetables is better to be consumed in the time of the outbreak. This process can be done with the help of media and authorities. Also in this process a facilitator can play a role in encouraging the different stakeholders to participate and share knowledge. The facilitator can help people in the process of facilitation to better negotiate in decision making. Collective and participatory research and learning would enable the producers and retailers to accept new knowledge (Groot et al., 2000).

In food safety issues government has a leading role; it can mobilize and inspire other actors such as media and industry. The role of government is not just to enforce the change, but to inspire the process of learning and encourage the other actors to think and take part (Rotmans et al., 2001). It can start using companies and suppliers as their partners to enable them to decide properly. Strong connections are needed between authorities, suppliers and retailers on issues relating to food safety management. Furthermore it can play a role in educating and informing people. Establishing education programs to give general information in food safety issues to consumers can be done at different levels such as the school education system, at home, in the workplace, through education for food professionals and also through the media (Wallas et al., 2007).

In a broader perspective the responsibility of governments is therefore to provide a facilitating institutional and regulatory environment for food control (Fan et al., 2009). On the other hand increasing food control will also increase the price of the products. So the question to be answered is that if the consumers are willing to pay more for foodstuff that has been produced in this way. And if distributors and retailers are to pay a higher price to growers of more secure produce.

According to the interviews no one seems willing to take the responsibility of the socio-economic effects of *E. coli* outbreak in the time of outbreak. The result of interviews with food quality managers show that they blame media and authorities for the loss which they have had. Consumers on the other hand think that more monitoring and less import from other countries could have helped.

Consumers should be aware of their role in food safety. According to Hall et al., (1997) "Approximately 85% of all outbreaks occur as a result of food mishandling in food service establishments or homes." Therefore consumers should take responsibility in this area. It seems that the only source of information for consumers regarding food safety is media. A survey which was conducted in 1995 revealed that participants mostly (71%) get food safety information from television and newspapers (Oger et al., 2001). Consumers should be more active in searching and receiving information from more reliable sources than media regarding food safety issues. In future study it would be good to ask consumers about their idea of consumer responsibility.

Generally the role which media plays is "calling public attention to food safety issues." Public trust is an important issue regarding food safety and media have a responsibility to report true and fact-based information to customers (Fan et al., 2009). In the time of outbreak in 2011 people have been informed about the EHEC problem through the media but they do not have any other information which can be helpful to them regarding the consequences of it. Generally the food safety information which is offered to consumers through the media should go to a deeper level. Selling papers should not be the only aim for a newspaper since this can result in sensationalistic and unreliable coverage.

Media can give people relevant information about safe food handling practices or food hygiene on the news or even in TV cooking programs (Fan et al., 2009). For instance "Halvåtta hos mig" that many people watch if it had three minutes about food safety, it would have been good. Giving indirect food safety education to the public through popular TV series or movies is another way of educating people through the media. Providing information and advertisements about food safety related guidelines on the web is another role which the media can undertake. Also the collaboration between the media and industry works quite well. Media can produce programs or manage press conference which shows how producers or industry work or manage the problem in the time of the outbreak.

## 5. Conclusion

To deal with food safety issues there is a need for a broad view since food safety is a result of the interaction of several factors. Wang and Ahmed (2003) claim that "changes in behaviour and lifestyle, as needed for sustainable development, also require the unlearning of existing beliefs and methods that might otherwise hinder the new learning needed."

In order to increase produce safety through cultural management, further study on the interaction of bioactive compounds and different strains of *E. coli* is needed. To be able to apply the results of laboratory work at a farm level; strong collaboration between governments, suppliers, industry, media and scientists is needed. Better intervention of government and more fact-based news coverage in media about EHEC outbreak would help both producers and consumers. Therefore a "transition" which occurs in different areas such as culture, belief systems, policy action, along with new methods in agriculture is needed to manage the problem in future.

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# 7. Appendix:

**Appendix 1:**

**Interview with food quality managers**

1- Did the *E. coli* outbreak affect your business last year? How was the organizations' reaction to it?

2- How do you design safety into a food product or food process in your organization?

-Water activity, PH, or chemical food preservative

-Prevention of microbial growth

-Destruction of microorganisms

-Prevention of contamination

3- Could you please tell me which method you follow in your food safety quality control? Do you know about the HACCP method? Have you ever applied HACCP in the process of quality control in your organizations?

4- What do you think is the role of risk assessment and management in the recognizing and managing of foodborne disorders? Could you please tell me about the application of risk assessment and management in your company?

5- In order to build proper food safety management what are the essential steps and practices in your opinion?

6- Industry and market and media must collaborate in order to build more trusting relationship. How do you evaluate the role of media at the time of an outbreak? What do you think that media should do at this time?

7- Do you consider educating and talking to the consumers in your organization? As the retailors and food service providers you have the opportunity to talk directly to consumers; how do you see the role of education in consumer's attitude toward food safety issues?

8- Is there any specific guideline which has been published by your organization or any other organizations in this area after the E. coli outbreak?

9- What do you consider the government's role in food safety issues?

**Appendix 2:**

**Interview with consumers in Swedish**

Konsumentenkät om tarmsmittor på frukt och grönt

Kön kvinna □ man □

Ålder: 20-30 □ 31-40 □ 41-50 □ 51-60□

61-70 □ 71-80 □> 80 □

1) Känner du till EHEC-utbrottet som skedde förra vår/sommar?

Ja □ Nej □

2) Känner du till EHEC's inverkan på hälsan?

Ja □ Nej □

3) Känner du någon som insjuknade i EHEC i samband med utbrottet 2011?

Ja □ Nej □

4) Hur påverkades din konsumtion av frukt och grönt vid tidpunkt för utbrottet?

a. Inte allts □

b. Jag köpte inga grönsaker ett tag □ några veckor □

månader □

5) Vilka grönsaker konsumerar du mindre sedan fjolårets utbrott?

Gurka □ Spenat □

Sallad □ Groddar □

Andra grönsaker som inte nämnts ovan? _____

6) Hur har fjolårets utbrott i Tyskland påverkat dina konsumtionsvanor av frukt och grönt?

_____

_____

7) Vilka förslag har du till odlare och handeln för att förbättra situationen i framtiden?

a) Mer övervakning i distributionssystemet □

b) Mindre import av frukt och grönt från utlandet □

c) Striktare lagar □

d) Mer information om EHEC utbrott i medier □

TACK FÖR DIN MEDVERKAN!

**Appendix 3:**

**SPSS Tables**

**Table 1:**

Participation according to age

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | 20-30 | 36 | 36.0 | 36.4 | 36.4 |
| | 31-40 | 32 | 32.0 | 32.3 | 68.7 |
| | 41-50 | 15 | 15.0 | 15.2 | 83.8 |
| | 51-60 | 5 | 5.0 | 5.1 | 88.9 |
| | 61-70 | 7 | 7.0 | 7.1 | 96.0 |
| | 71-80 | 4 | 4.0 | 4.0 | 100.0 |
| | Total | 99 | 99.0 | 100.0 | |
| Missing | system | 1 | 1.0 | | |
| Total | | 100 | 100.0 | | |

**Table 2:**

Participation according to gender

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | Female | 33 | 33.0 | 41.3 | 41.3 |
| | Male | 47 | 47.0 | 58.8 | 100.0 |

| | | | | | |
|---|---|---|---|---|---|
| | Total | 80 | 80. 0 | 100.0 | |
| Missing | System | 20 | 20.0 | | |
| Total | | 100 | 100.0 | | |

**Table 3:**

Have people heard about EHEC outbreak?

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | yes | 84 | 84.0 | 84.0 | 84.0 |
| | no | 16 | 16.0 | 16.0 | 100.0 |
| | Total | 100 | 100.0 | 100.0 | |

**Table4:**

Do the respondents know about EHEC consequences on people's health?

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | yes | 46 | 46.0 | 46.0 | 46.0 |
| | no | 54 | 54.0 | 54.0 | 100.0 |
| | Total | 100 | 100.0 | 100.0 | |

**Table 5:**

Do the respondents know anyone who became sick due to the outbreak?

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | Yes | 38 | 38.0 | 38.4 | 38.4 |
| | No | 61 | 61.0 | 61.6 | 100.0 |
| | Total | 99 | 99.0 | 100.0 | |
| Missing | System | 1 | 1.0 | | |
| Total | | 100 | 100.0 | | |

**Table 6:**

Did the EHEC outbreak affect people's food habits?

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | did not affect | 64 | 64.0 | 64.0 | 64.0 |
| | Affect | 36 | 36.0 | 36.0 | 100.0 |
| | Total | 100 | 100.0 | 100.0 | |

**Table 7:**

For how long were consumers' food habits affected by the outbreak?

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | for a while | 25 | 25.0 | 62.5 | 62.5 |
| | couple of weeks | 13 | 13.0 | 32.5 | 95.0 |
| | months | 2 | 2.0 | 5.0 | 100.0 |
| | Total | 40 | 40.0 | 100.0 | |
| Missing | System | 60 | 60.0 | | |
| Total | | 100 | 100.0 | | |

**Table 8:**

Vegetables which have been consumed less after the EHEC outbreak

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | Cucumber | 12 | 12.0 | 28.6 | 28.6 |
| | Lettuce | 10 | 10.0 | 23.8 | 52.4 |
| | Spinach | 8 | 8.0 | 19.0 | 71.4 |

| | | | | | |
|---|---|---|---|---|---|
| | Sprouts | 8 | 8.0 | 19.0 | 90.5 |
| | cucumber lettuce | 1 | 1.0 | 2.4 | 92.9 |
| | spinach sprouts | 1 | 1.0 | 2.4 | 95.2 |
| | cucumber lettuce sprouts | 1 | 1.0 | 2.4 | 97.6 |
| | cucumber lettuce spinach sprouts | 1 | 1.0 | 2.4 | 100.0 |
| | Total | 42 | 42.0 | 100.0 | |
| Missing | System | 58 | 58.0 | | |
| Total | | 100 | 100.0 | | |

**Table 9:**

Respondent's suggestion for improving the situation

| | | Frequency | Percentage | Valid Percentage | Cumulative Percentage |
|---|---|---|---|---|---|
| Valid | More monitoring | 20 | 20.0 | 21.7 | 21.7 |
| | Less import | 20 | 20.0 | 21.7 | 43.5 |
| | Stricter law | 14 | 14.0 | 15.2 | 58.7 |
| | More info on media | 7 | 7.0 | 7.6 | 66.3 |
| | monitoring & media | 3 | 3.0 | 3.3 | 69.6 |

| | | | | | |
|---|---|---|---|---|---|
| | monitoring & law | 9 | 9.0 | 9.8 | 79.3 |
| | | 6 | 6.0 | 6.5 | 85.9 |
| | Less import and law | 2 | 2.0 | 2.2 | 88.0 |
| | Less import and law | 6 | 6.0 | 6.5 | 94.6 |
| | law & media | 2 | 2.0 | 2.2 | 96.7 |
| | More monitoring& Less import& Stricter laws | 1 | 1.0 | 1.1 | 97.8 |
| | monitoring law media | 1 | 1.0 | 1.1 | 98.9 |
| | All answers | 1 | 1.0 | 1.1 | 100.0 |
| | Total | 92 | 92.0 | 100.0 | |
| Missing | System | 8 | 8.0 | | |
| Total | | 100 | 100.0 | | |

**Table 10:**

Case Processing Summary

| | Cases | | | | | |
|---|---|---|---|---|---|---|
| | Valid | | Missing | | Total | |
| | N | Percentage | N | Percentage | N | Percentage |

| | | | | | | |
|---|---|---|---|---|---|---|
| affect * age * gender * sick * consequences | 79 | 79.0% | 21 | 21.0% | 100 | 100.0% |
| time * age * gender * sick * consequences | 26 | 26.0% | 74 | 74.0% | 100 | 100.0% |
| veg * age * gender * sick * consequences | 28 | 28.0% | 72 | 72.0% | 100 | 100.0% |
| suggestion * age * gender * sick * consequences | 71 | 71.0% | 29 | 29.0% | 100 | 100.0% |

Symmetric Measures

| Age | | | | Value | Asymp. Std. Error | Approx. T[b] | Approx. Sig. |
|---|---|---|---|---|---|---|---|
| 20-30 | Nominal by Nominal | Phi | | 0.242 | | | 0.201 |
| | | Cramer's V | | 0.242 | | | 0.201 |
| | Ordinal by Ordinal | Gamma | Zero-Order | 0.613 | 0.367 | 1.437 | 0.151 |
| | N of Valid Cases | | | 28 | | | |
| 31-40 | Nominal by Nominal | Phi | | 0.346 | | | 0.072 |
| | | Cramer's V | | 0.346 | | | 0.072 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ordinal by Ordinal | Gamma | Zero-Order | 1.000 | 0.000 | 2.278 | 0.023 |
| | N of Valid Cases | | | 27 | | | |
| | Nominal by Nominal | Phi | | 0.158 | | | 0.569 |
| | | Cramer's V | | 0.158 | | | 0.569 |
| 41-50 | | | | | | | |
| | Ordinal by Ordinal | Gamma | Zero-Order | 1.000 | 0.000 | 0.987 | 0.323 |
| | N of Valid Cases | | | 13 | | | |
| | Nominal by Nominal | Phi | | .[c] | | | |
| 51-60 | | | | | | | |
| | N of Valid Cases | | | 2 | | | |
| | Nominal by Nominal | Phi | | 0.632 | | | 0.121 |
| | | Cramer's V | | 0.632 | | | 0.121 |
| 61-70 | | | | | | | |
| | Ordinal by Ordinal | Gamma | Zero-Order | 1.000 | 0.000 | 1.309 | 0.190 |
| | N of Valid Cases | | | 6 | | | |
| | Nominal by Nominal | Phi | | .[d] | | | |
| 71-80 | | | | | | | |
| | N of Valid Cases | | | 4 | | | |
| | Nominal by Nominal | Phi | | 0.281 | | | 0.012 |
| Total | | | | | | | |
| | | Cramer's V | | 0.281 | | | 0.012 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ordinal by Ordinal | Gamma | Zero-Order | 0.814 | 0.181 | 2.950 | 0.003 |
| | | First-Order | 0.788 | | | |
| | | Partial | | | | |
| N of Valid Cases | | | 80 | | | |

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. No statistics are computed because hear and gender are constants.

d. No statistics are computed because hear is a constant.