

Coordination between school and university for the experimental work on determination of level of antioxidants in food samples and drinks using Briggs-Rauscher reaction and TRL/luminescence fingerprinting

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The actual theme of this thesis is to develop co-operation between schools and university and hence to provide the students an opportunity to utilize the university laboratory equipment in the experimental work. This research work is based on developmental techniques in which the experimental work was first tried with the first group of secondary school students in OpiLUMA laboratory of the chemistry department of University of Turku and was later modified for upper secondary school students according to their level.

Literature review helped to discover/select the experimental methods to be used in this context. The data collected through questionnaires during the experiments was later analysed to see the learning outcomes and interest of the students visiting OpiLUMA laboratory. The international and Finnish curriculum books were thoroughly analysed using content analysis technique to investigate if the topic was explained well highlighting the importance of antioxidants in daily diet.

The main objective of this research is to develop the laboratory work for "determination of level of antioxidants in food and drinks". It started with the literature review to investigate the work done previously in this context and then check the objectives of the chemistry curriculum outlined by Finnish National Board of Education. Experimental work was developed based on the observations and the feedback obtained from the first trial with the first group of visiting students. Feedback was reported in the form of questionnaires filled by the students expressing their interest in the topic and hoping for more coordination between the university and the school.

The school teacher of the visiting students also considered such co-ordination important and beneficial for the students as students got an opportunity to utilize the OpiLUMA laboratory equipment, which is usually not available in school laboratories.

Students learnt about the importance of taking antioxidants regularly in daily diet. Briggs-Rauscher reaction was more interesting for all students due to series of colour changes involved which could very easily help them to know the relative concentration of antioxidants in the food samples. Luminescence fingerprinting used for testing adulteration in food was totally new technique introduced to upper secondary school students. These techniques proved to be very fruitful to make chemistry relevant to life. Content analysis of the books revealed that the topic of antioxidants has not been described well in Finnish curriculum as in the international curriculum books.

This research work finds the coordination between schools and university to be a powerful mean to develop students' interest in chemistry by making chemistry more relevant to life, hence motivating them to take more chemistry courses.

Keywords: chemistry, antioxidants, co-ordination, experimental work, research

Contents

1	Introduction	1
1.1	Main objectives of chemistry learning.....	1
1.2	Project specific objectives	2
1.3	The content and the chapters	2
1.4	Structure of the chapters	3
2	Chemistry teaching and learning.....	4
2.1	PCK Pedagogical Content Knowledge.....	5
2.1.1	History	5
2.1.2	Modern pedagogies.....	5
2.2	How to allocate chemistry curriculum between science and society	5
2.3	Preparing for scientific literacy for all	6
2.4	Learning for understanding.....	6
2.5	Learning as information processing	6
2.6	Setting up objectives for Chemistry teaching.....	8
2.7	Guiding principles for structuring the whole curriculum	9
2.8	Interest and motivation for chemistry learning	9
2.9	Chemistry teaching and learning in upper secondary schools	9
2.10	Project specific objectives to be fulfilled in accordance with Finnish Curriculum.....	10
3	Relevance	11
3.1	Definition.....	11
3.2	Different forms of relevance	12
3.3	Different ways to relate everyday chemistry to the school curriculum	12
3.4	Food for thought	13
3.5	Pedagogical recommendations for attaining chemical literacy	15
4	Introduction to free radicals and antioxidants.....	16
4.1	Free Radicals:	16
4.1.1	Endogenous sources of free radicals.....	17
4.1.2	External sources of free radicals.....	17
4.2	Antioxidation and antioxidants	19
4.2.1	The body's antioxidant defence	19
4.2.2	Chain breaking antioxidants.....	20
5	Research methods used for content analysis.....	21
5.1	Qualitative Content analysis.....	21
5.1.1	What is content analysis?.....	21
5.1.2	Difference between inductive and deductive approach	21
5.1.3	History	22
5.2	Data management and analysis methods	22
5.2.1	Definition.....	22
5.2.2	Components of data analysis	23
5.2.3	Need to select data management process.....	25
5.2.4	General steps involved in data management process.....	25
5.2.5	Process of inductive data analysis as presented by Miles and Huberman	25
5.3	Stepwise process of inductive content analysis carried out in this research work	26
5.3.1	Preparation phase	26
5.3.2	Data collection.....	27
5.3.3	Formulation of categories while organizing qualitative data	28
5.3.4	Abstraction	29

6	Results of the content analysis of the course books	30
6.1	Content analysis of course books used in international curriculum	31
6.1.1	IB Chemistry book and study guide	31
6.1.2	IB biology books	32
6.2	Content analysis of course books used in Finnish curriculum	36
6.2.1	TERVE 1	36
6.2.2	TERVE 2	37
6.2.3	KEM 1, KEM 2 and KEM 3	37
6.2.4	BIOS 1, BIOS 2 and BIOS 3	37
6.2.5	Koralli 1,2 and 3	38
6.2.6	Mooli 1 (experimental work)	38
6.2.7	Mooli 2 (experimental work)	38
6.2.8	Tarmo 1 and 2	38
7	Research method for case study used for experimental work	39
7.1	Definition	39
7.2	Strengths and weaknesses of the method	40
7.3	Types of case study	40
7.4	Data required for case study	41
8	Results of the case study conducted in present research work	41
8.1	Data regarding age and sex of the participating students	41
8.1.1	Data collected from the questionnaires	43
8.1.2	Data regarding the most interesting part of the planned experimental work	45
8.1.3	Feedback by the students regarding experimental work	46
9	Discussion;	46
9.1	Experimental work designed for the research	46
9.2	Scientific literacy for all	47
9.3	Moving from known to unknown	48
9.4	Relevance	48
9.5	Inductive content analysis	49
9.6	Selecting the suitable research method	49
9.7	Process of data management	50
9.7.1	Preparation phase	50
9.7.2	Data collection	50
9.7.3	Organizing qualitative data	50
9.7.4	Categorization	51
9.7.5	Abstraction	51
9.7.6	Reducing the data	52
10	Conclusions	53
10.1	Description of antioxidants in the course books	53
10.2	Relevance of chemistry teaching with the help of antioxidants	53
10.3	Reliability of the research and further research ideas	55
	References	56

1 Introduction

This research work was aimed at defining relationship of chemistry to everyday life as well as the interrelationship of chemistry with other branches of science. It is aimed to create long-lasting connection between chemistry and real life, hence to motivate the students to choose more chemistry courses in upper secondary schools and universities. In Finland, one of the objectives included in curriculum of first compulsory chemistry course, *Kemia kaikiällä*, in upper secondary schools is to provide students with experiences to stimulate and deepen their interest in chemistry, so as to improve their skills to apply knowledge of chemistry in everyday life. The main goal of teaching chemistry is to guide the students in identifying their chemistry skills and set their own learning goals to meet the learning challenges (Opetushallitus, 2015, p 157-160).

In many countries, studies have been carried out to investigate the decline in the number of students studying chemistry at the university level. Out of 25 countries involved in an investigation, some countries including Finland were found to have successful educational systems according to international tests like PISA and TIMSS, but most of the students were found to have negative opinions about chemistry. It is, therefore, needed to investigate what could be done to improve the current situation to improve students' participation in chemistry courses (Broman et al, 2011). In an analysis carried out in Sweden, students suggested making chemistry relevant to everyday life and being more practical and more student centred (Broman and Simon, 2015).

Teaching strategies are helpful to induce inspiration for learning by guiding students towards characteristic scientific thinking, use of information, ideas, interaction and assessment of reliability and relevance of knowledge in different situations (see appendix, report of the research work).

1.1 Main objectives of chemistry learning

While planning the research work, the main objectives of chemistry had to be kept in mind which include development of scientific thinking, understanding importance of chemistry and its applications in their lives, living environment, society and technology to safeguard environment and human well-being (Opetus hallitus, 2014).

1.2 Project specific objectives

Finding antioxidants as topic of great interest for the scientists related to food chemistry, environmental chemistry and many other fields of science, this topic was selected for the present research work. As this topic can be very well used to show integration of different branches of science as biology, chemistry, biochemistry and food chemistry as well as relevance of chemistry to everyday life, it could be likewise used for chemistry as well as biology teaching in secondary and upper secondary schools. It might help to attract students' interest by relating it to daily food intake as well as biological systems.

The main goal was to focus on the macroscopic level of antioxidants as this topic has not been mentioned well in the secondary school chemistry and biology course books, though health education books say a little about antioxidants which is also insufficient.

Considering the importance of chemistry as a practical subject, school students were provided with the opportunity to work in the scientific environment using more advanced laboratory equipment which could be helpful to attract students' interest in chemistry subject. Using Briggs-Rauscher reaction, the activity was planned to determine the antioxidant level in different food samples in the very first step. Later part of the research work was development of the previous work for the upper secondary school students by adding luminescence fingerprinting of food products.

1.3 The content and the chapters

The aim of this research work is to present the essential knowledge which can be used by a chemistry teacher in her or his daily practice. This research work certainly does not include a review of all the course books available in international and Finnish curriculum, neither it contains all the research findings available regarding teaching of chemistry, but one can find the most prominent and important issues while dealing with teaching chemistry in secondary and upper secondary school.

This research work is an attempt to change the way chemistry is being taught in secondary and upper secondary schools leading students to consider chemistry not relevant to daily life, hence not interesting. By explaining the different levels of chemistry knowledge through literature review, it is suggested to plan the current courses of chemistry accordingly to implement student based curriculum by deciding which information to which level is needed for the specific age group of students.

While taking these decisions, students' interest should be primarily in mind. Student-centred activities should be essential component of the teaching plan which incorporate inquiry-based approaches through student laboratory work and cooperative learning methods.

1.4 Structure of the chapters

All the chapters explain the pedagogical aspects of teaching and learning chemistry in a systematic way. Every chapter provides an overview of different issues in chemistry teaching. Most of the essential concepts regarding learning and teaching chemistry observed during the research have been mentioned to help the chemistry teachers to enrich their knowledge by following ideas which might be suitable to their own teaching classrooms.

First chapter, *introduction*, basically highlights the background and main objectives to carry out this research work. It presents an overview of the content and the structure of the chapters included.

Chapter 2, *Chemistry teaching and learning*, narrates the history of pedagogical content knowledge and modern pedagogies. It provides suggestions to how to allocate chemistry curriculum between science and society leading to learning for understanding, thus providing scientific literacy for all.

By presenting different levels of chemistry knowledge, it suggests the guiding principles for structuring such a curriculum which motivates the students to learn more chemistry in upper secondary schools and universities.

In chapter 3, *relevance*, it is attempted to address the issue of relevance which is the question under discussion in this research work. By taking prominent references from literature review, this chapter portrays different forms of relevance and the different ways to implement the phenomenon to chemistry courses in secondary and upper secondary schools. Based on evidences and examples from curricula, it suggests pedagogical recommendations for attaining chemical literacy.

Chapter 4, *introduction to free radicals and antioxidants*, gives knowledge about free radicals on the macroscopic level connecting it somehow to the microscopic level.

Chapter 5, *research methods used for content analysis*, provides a closer look at the stepwise process of qualitative inductive content analysis as well as defines the data management and analysis methods in detail. It also explains the process of

inductive data analysis as suggested by Miles and Huberman (1994). Furthermore, it gives an overview of the stepwise process used in this research work.

In chapter 6, *results of the content analysis of the course books*, all the data generated during content analysis of the curriculum books is reported in the mind maps. A closer look at the data indicates how the topic of antioxidants has been treated in the international as well as Finnish curriculum.

Chapter 7, *research method for case study approach*, provides pictorial demonstration of the types of case study starting with its definition and illustrates the strengths and weaknesses of the research method. Furthermore, it describes the data required for the case study.

In chapter 8, *results of the case study conducted in present research work*, all the data collected during experimental work has been presented in the form of tables and graphs giving valuable information about the process. It also includes the feedback by the students to evaluate the outcome of the learning process.

In chapter 9, *discussion*, the whole research work is analysed based on evidences from literature and data gathered during content analysis as well as the observations carried out during the experimental work designed for the students.

Chapter 10, *conclusions*, portrays the extract of all the process and methods used in the research conducted and provides ample support for the assertion that further research in this area is needed to create harmony and long-lasting relationship between chemistry and other branches of science.

2 Chemistry teaching and learning

Learning chemistry as a science not only helps to develop the interaction of science, life and society but also many skills as problem-solving, thinking in models, being aware of hazards and even to develop general educational skills. Chemistry education is not only beneficial for career opportunities in chemical industry but understanding chemistry is needed in almost all other science-related fields like biology, geology, environmental sciences, archaeology and medicine. All these fields could benefit from good chemistry education on high school level, knowledge of chemistry and its current issues.

2.1 PCK Pedagogical Content Knowledge

2.1.1 History

In 1960s and early 1970s most of the chemistry programmes were based on conceptual approach to chemistry but currently based on the philosophy that the curriculum should place more emphasis on students' interests and motivation.

Magnusson et al in 1999 defined PCK to include five components (adopted from general science teaching to chemistry teaching): (cited by Eilks and Hofstein, 2013).

- Orientation towards chemistry teaching to include goals for and approaches to teaching chemistry
- knowledge of the chemistry curriculum
- knowledge of instructional techniques in chemistry, i.e. pedagogy
- knowledge of assessment methods in chemistry
- knowledge of students' understanding of chemistry

2.1.2 Modern pedagogies

Modern pedagogies of chemistry learning should encompass student-centred activities and inquiry-based approaches should be incorporated in all the educational systems independent of level of development through student laboratory work, co-operative learning methods and the support of ICT for enhancing achievement.

Vesterinen and Aksela suggest;

To make informed curricular decisions, there is a need to teach future chemistry teachers, not only ontological chemical knowledge, but also philosophical and sociological perspectives of chemical practice and reflection on the role of chemistry in society. To address nature of chemistry related issue, there is a need to include discussion on the NOS related topics and teacher-scientist interaction to chemistry teachers' education (Vesterinen and Aksela, 2009).

2.2 How to allocate chemistry curriculum between science and society

Chemistry curricula can use different approaches towards motivation to the learning of chemistry. Curricula have become over-loaded with content due to massive accumulation of scientific knowledge. High content loads make the curricula too often aggregations of isolated facts detached from their scientific origin (Eilks et al,2013).

2.3 Preparing for scientific literacy for all

Since 1980s, concept of “scientific literacy for all” brought acknowledgement that generally basic understanding of science and particularly understanding of chemistry is needed for all (Eilks et al, 2013).

New standards demanded chemistry education to contribute to general educational objectives. PISA (Program for International Student Assessment), in terms of science education to all, demanded scientific literacy to be the capacity to use scientific knowledge, to understand and help make decisions about the natural world and the change made to it through human activity (cited by Shwartz et al, 2013).

2.4 Learning for understanding

Main goal of chemistry education research is to understand and improve chemical learning and teaching. To make students learn science concepts with understanding and for life involves experimental work, using different pictorial material, context-based approaches and multimedia environments. It is important to develop students’ chemical literacy to enable them to use their science knowledge in different real-life situations. (Devtak and Glazer, 2014).

Chemistry curriculums have changed over the decades from the traditionally oriented chemistry teaching emphasizing symbolic and mathematical components of chemical concepts to more context-based enquiry learning-oriented teaching supported by different applications of informational-communicational technology. It is important to develop students’ scientific/chemical literacy, so that they will be able to use their science knowledge in different real-life situations. On the other hand, teachers should be adequately educated so that they can efficiently implement curriculum innovations. (Devtak and Glazer, 2014).

2.5 Learning as information processing

How to teach chemistry to reduce the difficulties in understanding? Johnstone’s (1999) insight into chemistry learning is as follows (cited by Reid, 2014, p 86-7).

He described three forms of activities in chemistry.

- 1) Macrochemistry; to see colour, detect smells, observe reactions and describe materials.

- 2) Micro level is world of atoms, molecules, bonds, electrons, and so on. (not easily accessible to senses)
- 3) Symbolic level; chemists present the world of chemistry by means of formulae, equations, diagrams, and mathematical representations.

An experienced chemist can move happily at all the three levels but a learner simply can't do it as amount of information is more than limited working memory capacity. Johnstone (1999) pictured it in terms of triangle (Fig 1);

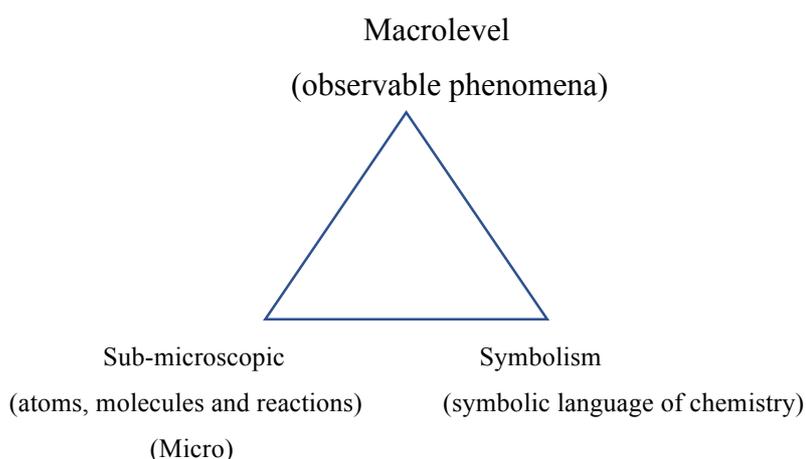


Figure 1. Three levels of chemistry knowledge

Johnstone (1999) argues that early chemistry courses must be dealing with macro, the descriptive level. When the school students become more familiar with the descriptive terms, the micro level can be gently introduced as a way of explaining why macro-chemistry takes place in the way it does. The symbolic must be introduced carefully, always ensuring that it is perceived as a way of simplifying the previous knowledge (cited by Reid, 2014, p.86-7).

Tsaparlis (2014) also discusses the chemistry as a multi-representational structure by explaining the great difficulties faced by the students while trying to grasp concepts at the sub-micro level. Using active-learning methodology properly in teaching and properly linking the macro with the sub-micro levels, teachers can contribute to meaningful learning and conceptual understanding of the particulate concepts of matter (Tsaparlis, 2014, pp. 41-61).

Finnish curriculum for upper secondary school chemistry goes in parallel to meet the three levels of chemistry knowledge (Opetus hallitus, 2015, pp.157-160).

Kemian opetus tukee opiskelijoiden käsitteiden rakentumista sekä ilmiöiden ymmärtämistä siten, että niiden makroskooppinen, mikroskooppinen ja

symbolinen taso muodostavat loogisen kokonaisuuden. Opiskelijoiden aikaisemmista kokemuksista ja havainnoista edetään ilmiöiden kuvaamiseen ja selittämiseen sekä aineen rakenteen ja kemiallisten reaktioiden mallintamiseen kemian merkkielellä ja matemaattisesti.

Chemistry lessons in school usually advance very quickly to the subatomic level of matter to electrons and protons, and stoichiometric relationships. This is far away from the macroscopic level which can be directly perceived, e.g. colour, stickiness, elasticity or brittleness, transparency (like ice), or turbidity (like snow). All these properties are the result of the molecular and supramolecular structure and order of the material. It therefore seems to be promising to proceed from “large” to “small”- from macroscopic to molecular level- and thus not to lose sight of the property-structure relationship this could also help in the perception of the chemistry and physics as basic principles of the substantial world and of life, irrespective of whether materials are natural or artificial, instead of treating chemistry as an abstract and difficult topic (Mischnick, 2011).

2.6 Setting up objectives for Chemistry teaching

Keeping in mind the age, goals, expectations, motivation and capacities of the students, teachers will be able to set more reliable goals. Content knowledge may be specified by a national or regional syllabus, a specific textbook or by the teachers themselves. Teacher should know the purpose of teaching, the content knowledge as well as depth and breadth of the scientific detail of the content.

Goals regarding thinking skills and scientific practices could be set by using Bloom`s revised taxonomy (cited by Shwartz et al,2013, see Fig 2).

High order thinking skills

Creating
Evaluating
Analysing
Applying
Understanding
Remembering



Lower order thinking skills

Figure 2; Bloom`s revised taxonomy

Bloom(1956) suggests that,

Students can use the concepts, ideas and information presented in the chemistry course in various levels of thinking: they can simply memorise and remember, understand the meaning, apply to a different context, analyse a complex phenomenon, or investigate the relationship between concepts, evaluate the validity of an argument, the quality of experimental data, and the limitations of a specific model, etc. At the highest level, students create their own pattern, structures and generalisations (cited by Shwartz et al, 2013).

2.7 Guiding principles for structuring the whole curriculum

De Jong (2006) differentiated four different domains to be utilized towards learning of chemistry as guiding principles for structuring the whole curriculum (cited by Eilks et al, 2013).

- 1) The personal domain; connecting chemistry with the student's personal life.
- 2) The professional practice domain; providing information and background for future employment.
- 3) Professional and technological domain; enhancing the students' understanding of science and technological applications.
- 4) Social and society domain; helping the students to become responsible students in future.

2.8 Interest and motivation for chemistry learning

“Fundamental chemistry” driven curricula brought these facts which are also mentioned in PISA studies;

- 1) Lack of motivation among students
- 2) Lack of success in applying the learned concepts

2.9 Chemistry teaching and learning in upper secondary schools

Chemistry education in secondary school is often seen as divorced from real life due to use of abstract concepts and unfamiliar language in classrooms, hence students are unable to see the relevance, interest or importance of the material studied at school for their everyday life.

Chemical literacy is needed to understand, evaluate and make decisions about many current and future issues as nuclear power. School chemistry needs to help students understand and use basic chemical concepts but also to relate these concepts to real-world issues and show how chemistry helps in understanding and dealing with science-related issues of everyday life.

The goal is to explore the basis of “everyday chemistry” and its relation to chemistry content of secondary level courses. In the first class, students meet the difficult concepts of the atoms, molecules, electronic structure and safety measures in the laboratory, use of apparatus with the unfamiliar names as burette, pipette and flask and use of chemicals with unfamiliar names. Lack of relevance to real life seems more for chemistry than physics and biology due to the way chemistry teachers introduce chemistry to the students (Childs et al., 2015).

Research suggests *moving from familiar to unknown; start with concrete and move to the abstract, start simple and build up the complexity*. It mostly happens opposite to it.

General and specific objectives defined for chemistry teaching by Finnish National Core Curriculum had to be kept in mind, while planning laboratory activity for secondary and upper secondary school students. The Finnish National Core Curriculum provides the frame where the schools construct their own specific curricula. General objectives include improvement in learning environment, the development of cooperation skills and strengthening of concepts.

Main objectives of chemistry include development of scientific thinking, understanding importance of chemistry and its applications in daily life, living environment, society and technology to safeguard environment and human well-being (see appendix, report).

2.10 Project specific objectives to be fulfilled in accordance with Finnish Curriculum

Chemistry learning specific objectives (Opetus hallitus, 2014) to be obtained through this research work are as follows;

T3; To guide the students to understand the importance of chemistry know-how in their own lives, the living environment and society. Present research work

gives information about understanding antioxidants and free radical reactions in living systems and importance of antioxidants in daily food intake.

T6; To guide the students to carry out experimental research in collaboration with others, and to work safely and consistently. Students perform the lab activity independently in groups. Safety instructions are given before starting the experimental work to avoid all the possible hazards.

T7; To guide the students to process, interpret and present the results of its own investigations, as well as to evaluate them and the entire research process. Results are noted down in the table and then graphs drawn to explain the results.

T14; To guide students in their understanding of the basic principles of the properties, structure and changes in the material substance. Basic properties of antioxidants and their defense activity against the harmful activity of free radicals is explained in the beginning through pictures in powerpoint and later in the ending lecture. Luminescence theory is explained through excitation of electron by absorbing energy and then de-excitation losing energy (See appendix).

Aims being set while planning experimental work were mainly to create long-lasting connection between chemistry and relevance and to adapt healthy eating through chemistry (see appendix).

This experimental work clearly defines the relationship between two main branches of science they are studying, biology and chemistry. In addition, it integrates different branches of chemistry like analytical chemistry, organic chemistry, material chemistry and biochemistry. All this material can even be used for students of health sciences like nursing and related subjects relevant to food chemistry (see appendix).

3 Relevance

3.1 Definition

Relevance is defined as the students' perception of the content being taught satisfies their personal needs, personal goals or career aims. During chemistry lessons, future needs, goals and career aims might not be in the mind of the students. Further questions arise as, relevant to whom, for what and who is deciding about it.

3.2 Different forms of relevance

Different aspects of potential relevance can be summed up as; (Eilks et al, 2013)

Relevance for the individual;

Meeting students' curiosity and interest or contributing to their intellectual skill development.

Relevance for future profession;

Offering orientation and preparation for further academic or vocational training for future professions.

Relevance for the society;

Understanding the interdependence and interaction of science and society.

Combination of all these dimensions is important for structuring the chemistry curriculum which may provide purpose of learning chemistry and its potential uses to the students (cited by Eilks et al, 2013, pp 1-32).

For justifying chemistry education, the term 'relevance' is currently used in debates about why so many students do not like or do not learn chemistry quite well, as they perceive chemistry lesson irrelevant to them.

The context of chemistry education demonstrates that students participate actively in studies if subject matter presented to them is perceived as useful and relevant than if it appears remote (Johnstone, 1981).

3.3 Different ways to relate everyday chemistry to the school curriculum

There are different ways of relating everyday chemistry to the school curriculum and to the interests of secondary school students. Fig 3 shows some of the possible areas of everyday chemistry (Childs et al, 2015).

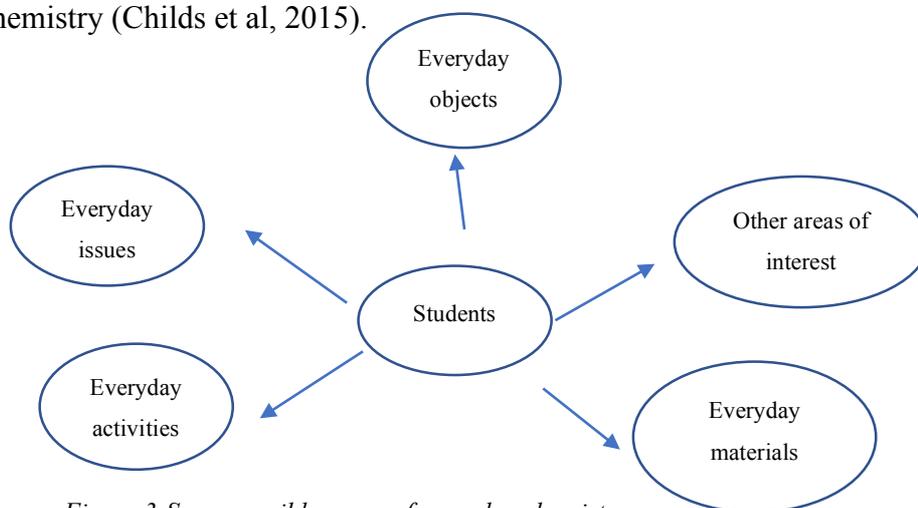


Figure 3; Some possible areas of everyday chemistry

Everyday material includes paints, paper, metals, clothing, insecticides and food etc. Everyday activities include keeping clean, cooking, transport, sport or entertainment. Everyday objects include the car, the paraglider, the bicycle, smart phones, laptop computers, space rockets etc.

3.4 Food for thought

Food is everyday contextual topic and is relevant to many chemistry topics in the secondary curricula. Using this simple context relevant to learners' everyday lives, idea may be extended towards physical properties of food (colours, smells, tests, cooking, changes etc.) by developing an understanding of the chemical concepts involved in these changes. Thus, the learner understands the macroscopic properties of food by developing their sub-microscopic understanding of chemical concepts.

Using food as context helps them understand chemistry, thus making them better-informed citizens. Table 1 explains the main sub-topics related to food, the concepts of secondary level chemistry that could be possibly taught relevant to food topic and then the possible related activities to be arranged by the teachers.

The context introduces the topic making the concept easy to understand. Such overlapping contextual links facilitate the development of a spiralling curriculum. New concepts introduced in a piece-meal 'need-to-know' fashion improve the clear understanding. e.g. the concept of isomers can be introduced through amino acids, lipids or citric fruits (Childs et al, 2015).

Table1. Food context, concepts and activities for teaching chemistry

Main sub-topics	Relevant topics	concepts	Ideas for possible activities
Food preservation nutrients	<p>Tinned fruits</p> <p>Macronutrients</p> <p>Proteins as natural polymers</p> <p>Enzymes (reaction in the body)</p> <p>Hormones(insulin)</p> <p>Binding proteins(haemoglobin)</p> <p>Amino acids</p> <p>Carbohydrates</p> <p>Lipids</p> <p>Food tests</p> <p>Iodine test for carbohydrates</p> <p>Biuret test for proteins</p> <p>Emulsion test for lipids</p> <p>Vitamin C</p>	<p>Denaturing of enzymes</p> <p>Polymers</p> <p>Functional groups of organic compounds</p> <p>Isomers</p> <p>Polar and non-polar compounds</p> <p>Carbohydrates; carbon, hydrogen and oxygen</p> <p>Lipids; saturated and unsaturated</p> <p>Hydrogenation of alkanes to alkenes</p> <p>Cis and trans isomers</p> <p>Use of starch as indicator in redox titrations</p> <p>Recognizing carbonyl functional groups</p> <p>Solubilities (polar & non-polar)</p> <p>Redox titrations</p>	<p>Investigation of the fresh pineapple, frozen pineapple, tinned pineapple on prepared jelly(gelatine)</p> <p>Understanding amino acids</p> <p>Non-polar side chains, polar chains, ionisable groups</p> <p>Compare leucine and isoleucine</p> <p>Investigation of fats:</p> <p>Comparison of saturated and unsaturated fats- use chemical composition and bonding to explain their properties</p> <p>Testing organic compounds:</p> <p>Burning foods to test presence of carbon</p> <p>Teacher demonstration: Carbon tower (sucrose and conc. Sulphuric acid)</p> <p>Carry out the test for starch on different carbohydrates: potato, bread, cereals, sugar</p> <p>Redox titrations where iodine is liberated</p> <p>Understand why the biuret test may give a colour change in the presence of a solution that is not a protein: What factor contributes to the colour change?</p> <p>Use emulsion test to explain that lipids are insoluble in water and soluble in ethanol</p> <p>Titration of vitamin C (ascorbic acid) with iodine to investigate the amount of vitamin C in different fruit juices.</p>

3.5 Pedagogical recommendations for attaining chemical literacy

Chemical curriculum at the high-school level should address the current goal of attainment of scientific literacy for all students and chemistry understanding levels should include the macroscopic, sub-microscopic, symbolic and process levels. Chemically literate students should have the ability to see the relevance and usability of chemistry in many related contexts and to attain it, following pedagogical recommendations are suggested (Shwartz et al, 2013).

Providing wide range of chemical ideas;

High-school introductory courses should provide wide scientific vocabulary and in addition to the concepts related to the structure of matter, many other ideas and concepts may be introduced leaving the calculation part for the advanced levels.

Decrease the domination of the chemical language;

Verbal expression should be valued more than exercising symbols to reduce the difficulties related to chemical symbols for non-science-oriented students.

Promoting understanding the nature of science;

Throughout the learning sequence, nature of science should be introduced to propagate scientific inquiry process.

Increased perception of relevance of chemistry studies;

Rather than focussing on knowledge of chemical facts, learning skills should be developed which make students see clear relevance and importance of chemical knowledge in their lives.

Focus on the development of higher-order thinking skills;

Main goal for chemistry education at the secondary level should be the development of general educational skills so that high school graduates are able to look for scientific information, critically read it and present it when needed.

Variety of assessment methods;

Both formative and summative evaluation should be carried out continuously throughout the whole study period. It can be via multiple choice and open-ended

questions and should also include e.g. portfolio of laboratory reports and oral presentations by individuals or pair of students and later reflection by students.

4 Introduction to free radicals and antioxidants

“Oxygen paradox” is the term indicating that oxygen is vital for survival of aerobic organisms but toxic at the same time due to generating free radicals and oxidative stress. Free radicals are produced during respiration and other cellular processes. Living organisms produce water- and lipid-soluble antioxidants to neutralize highly reactive free radicals. A balance between oxidative stress and antioxidant defence of the body must be maintained for a healthy life, otherwise excess free radicals can damage various biomolecules in the body.

4.1 Free Radicals:

It is defined as, “*an atom or molecule containing one or more unpaired electrons that are capable of independent existence*” (Dasgupta and Klein, 2014). They are produced during normal respiration and cellular functions. Some common free radicals and oxidants playing important role in human physiology are shown in table 2.

Table 2. Common free radicals and oxidants encountered in human physiology

Free radicals	Oxidants
Superoxide anion radical	Singlet oxygen
Hydroxy radical	Ozone
Peroxyl radical	Hydrogen peroxide
Lipid radical	Hypochlorite
Lipid peroxyl radical	Nitrous acid
Lipid alkoxy radical	Peroxynitrous acid
Nitric oxide	Nitrous oxide
Nitrosyl cation	
Thiyl radical	
Protein radical	

There are two sources of free radicals; *Endogenous* (internal) and *exogenous* (external) sources.

4.1.1 Endogenous sources of free radicals

Free radicals mentioned in table 3 are endogenous.

Table 3. Endogenous sources of free radicals

Physiological process	Comment
Mitochondrial respiration	Essential process of life generating superoxide anion radical
Respiratory burst	Process in which phagocytes consume a large amount of oxygen during phagocytosis
Metal ions	Essential metal ions like copper and ferrous ion, react with Hydrogen peroxide to produce free radicals
Infection	Immune system trying to neutralize the attacking microorganisms with a burst of free radicals is endogenous source of free radicals

Respiratory burst is a source of endogenous free radicals. It is a process by which phagocytes as white blood cells consume a large amount of oxygen during phagocytosis reaction producing free radicals mostly superoxide and also hydrogen peroxide.

4.1.2 External sources of free radicals

External sources include polluted air containing fine particulates less than 10 μm in diameter. These particles having free radical activity cause risk of asthma attack, lung inflammation and damage to lung cells. As preventive measures, patients susceptible to harmful effects of air pollution and those with coronary heart diseases should use face masks or stay indoors on hot days, when free radicals containing particulates may be in air at the ground level. (cited by Dasgupta and Klein, 2014). Some external sources of free radicals are shown in table 4.

Table 4. External sources of free radicals

External sources	Comments
Air pollution	Exposure to polluted air may cause oxidative stress, increasing risk for asthma, cardiovascular diseases and lung cancer
Inorganic particles in air	Oxidative stress caused in people working in industry by ingestion of mineral dust containing quartz, silica and asbestos from the air
Tobacco smoking	Oxidants in tobacco smoke damage lungs causing lung cancer
Exposure to radiation	Oxidative stress is caused by exposure to excessive ultraviolet light, sun bathing and radiation treatment in cancer therapy

Free radicals in smoke cause significant health hazards even in the form of passive smoking. Cigarette smoking is the leading cause of preventable death in the United States and health damage caused by cigarette smoke involves oxidative stress.

Drugs like anticancer drugs, immune-suppressants and antiretroviral drugs can induce oxidative stress. Drinking alcohol beverages can also induce oxidative stress. Minor oxidative stress induced by moderate drinking of alcohol can be easily counteracted by body's antioxidant defence mechanism. In addition, red wine is full of antioxidants. However, alcohol abuse is a serious health hazard (cited by Dasgupta and Klein, 2014).

Industrial solvents like carbon tetrachloride, chloroform and benzene can induce oxidative stress.

Ultraviolet light present in sunlight is essential for the production of vitamin D but too much exposure to ultraviolet light in sunlight provokes light-induced oxidative stress of skin, causing skin damage even skin cancer.

Free radicals have an important physiological role.

- They help with cell growth and cell proliferation.
- Free radicals help with the cell division.
- Free radicals regulate redox balance of the cell.
- They help in signal transduction.
- They activate protein kinases that regulate gene functions.
- They regulate immune function.

4.2 Antioxidation and antioxidants

Antioxidation includes all the processes that slow down or stop free radical oxidation.

Antioxidation process includes;

- Scavenging radicals to prevent their propagation
- Enzymatic hydrolysis of ester bonds to remove peroxidised fatty acids from lipids.

An effective antioxidant must have two properties.

- Antioxidant should react rapidly with errant radicals, giving a new radical.
- New radical species should be so non-reactive and it will not attack other molecules in the vicinity.

4.2.1 The body's antioxidant defence

The body's antioxidant defence consists of both exogenous and endogenous compounds which can be classified into three broad categories;

1) Antioxidant enzymes; They are superoxide dismutase (SOD), catalase and peroxidases.

2) Chain-breaking antioxidants; They can be water-soluble or fat-soluble and interfere with the chain reactions initiated by the free radicals. e. g. carotenoids, flavonoids, and antioxidant vitamins.

3) Metal binding proteins; Important antioxidant proteins include ferritin, transferrin and ceruloplasmin.

All antioxidants are helpful for proper neutralization of oxidative stress. Here only chain breaking antioxidants are discussed as they were mostly dealt with in the research topic and experimental work.

4.2.2 Chain breaking antioxidants

They are small molecules capable of neutralizing free radicals by breaking the chain reaction initiated by free radicals. They act by donating an electron or by receiving an electron from a free radical species, thus converting it into a stable species.

They can be classified into two broad categories; water soluble and fat soluble.

The most important water-soluble antioxidant is vitamin C, known as ascorbic acid. Vitamin C is capable of scavenging superoxide, hydroxyl, and aqueous peroxy radicals, as well as other oxidants, including hydrogen peroxide and singlet oxygen. In this process, vitamin C is converted into dehydroascorbic acid, which eventually breaks down into oxalic acid. Uric acid present in human blood acts as an antioxidant.

The most important fat-soluble chain breaking antioxidant is vitamin E (tocopherols and tocotrienols). Carotenoids are also important lipid soluble antioxidants and the most common form is β -carotene. These antioxidants can also neutralize peroxyradicals as well as singlet oxygen. β -carotene is precursor of vitamin A, which also has antioxidant activity. Flavonoids are antioxidants found in plants and food as fruits and vegetables. Tea and coffee are efficient sources of flavonoids (cited by Dasgupta and Klein, 2014).

Fang et al, (2002) emphasized that,

Understanding free radical biology is necessary for designing an optimal nutritional counter measure against space radiation-induced cytotoxicity.(Fang et al, 2002)

There has been growing evidence showing that oxidation of biomolecules and cell injury is caused due to malnutrition or excess of certain nutrients (e.g. vitamin C). There have been evidences portraying the fact that dietary antioxidants are useful radioprotectors and play an important role in preventing many human diseases. (e.g. cancer, atherosclerosis, stroke, rheumatoid, arthritis, neurodegeneration and diabetes).

The knowledge of enzymatic and non-enzymatic oxidative defence mechanisms will serve as a guiding principle for establishing the most effective nutrition support to ensure biological safety of manned space missions. (Fang et al. 2002).

5 Research methods used for content analysis

5.1 Qualitative Content analysis

Content analysis is a method used either for qualitative or quantitative data and in an inductive or deductive manner, both of which contain three main phases; preparation, organizing and reporting. Inductive approach is to start with no previous studies of the phenomenon and deriving concepts from the data while deductive approach is to use the previous knowledge or theory in a new situation.(cited by Elo and Kyngäs, 2008)

5.1.1 What is content analysis?

Content analysis is a method of analysing written, verbal or visual communication messages. It was first used to analyse the newspapers, magazines, articles, advertisements and political speeches in the 19th century and its use has been growing steadily during the last few decades (cited by Elo and Kyngäs,2008).

Content analysis known as method of analysing documents is systematic research method and objective means of describing and quantifying phenomena. It helps to understand the data by testing theoretical issues. This approach helps to distil words into fewer content-related categories. It is assumed that when classified into same categories, words, phrases and the like share the same meaning.

Content analysis helps to make valid inferences from data to their context and thus helping to attain a condensed and broad description of the phenomenon. The outcome of the analysis is concept or categories describing the phenomenon which are then used to build up a model, conceptual map, conceptual system or categories.

5.1.2 Difference between inductive and deductive approach

Content analysis may be used in an inductive or deductive way depending upon the purpose of research. In absence of sufficient previous knowledge, inductive approach is recommended. The categories are derived from the data in inductive content analysis. Deductive approach is used for theory testing.

Inductive approach moves from specific to general in which particular instances are observed and combined into a larger whole or general statement while deductive approach moves from general to specific (cited by Elo and Kyngäs, 2008).

5.1.3 History

Content analysis was criticized in the quantitative field for being too simple for detailed statistical analysis and not sufficiently qualitative in nature (cited by Elo and Kyngäs, 2008). In the early days, differentiation of the content analysis was limited to classifying it primarily as a qualitative vs quantitative research method. Despite the criticism, it has been useful to researchers as it is content-sensitive method (Krippendorff, 1980) and flexible terms of research design. By applying content analysis, research material gets organized into condensed and clear format without losing content information as objective is to create interesting, clear and highly valuable information from scattered data (Tuomi and Sarajärvi, 2009).

Basic analytical method which can be used in all traditional qualitative research methods is content analysis. Researcher Timo Laine at Jyväskylä university, presented many years ago the structure of the process of qualitative analytical research (Tuomi and Sarajärvi, 2009).

5.2 Data management and analysis methods

Michael Huberman and Matthew Miles (1984) advance a sophisticated and comprehensive model of the process to show how codes, memos and diagrams can help a researcher work from field notes to some conceptual understanding of the phenomenon being studied. This framework is very compatible with the computer-assisted methods of analysis.

5.2.1 Definition

Data management is defined as the operations needed for a systematic, coherent process of data collection, storage and retrieval (Miles and Huberman, 1994).

These operations are aimed at ensuring:

A) high-quality, accessible data

(b) documentation of just what analyses have been carried out and

(c) retention of data and associated analyses after the study is complete.

5.2.2 Components of data analysis

This definition contains three linked sub-processes. (Miles and Huberman, 1994)

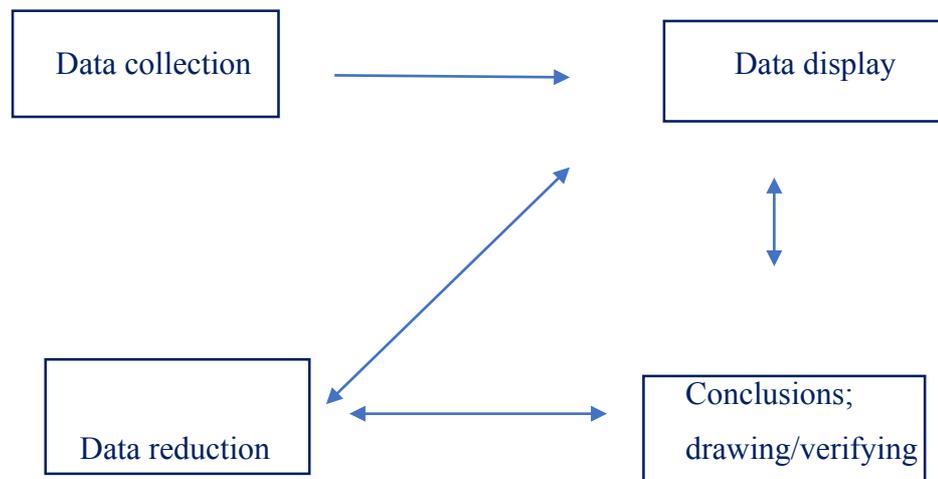


Figure 4. Components of data analysis; Interactive model

These processes occur during study design and planning before data collection, during early analyses while collecting data and then after data collection while completing final products.

Data reduction;

The potential universe of data is reduced and condensed by making data summaries, coding, finding themes, clustering and writing stories.

Data display;

It is an organized, compressed assembly of information leading to draw conclusions. It may include structured summaries and network like or other diagrams (cited by Miles and Huberman, 1994).

Conclusion drawing and verification;

It involves drawing meaning from the displayed data. Information is condensed, clustered, stored and linked over time, thus considered as “data transformation” (cited by Miles and Huberman, 1994).

Reducing the data in the analytical process;

Reducing the information to be analysed is condensing or splitting the data into parts. Expressions displayed in the original information are written down using expressions reflected from the reduced material and this phenomenon is termed as, “reducing the data”. An example described in content analysis applied during case study of a young

one receiving support regarding eating disorders is cited in Finnish literature (cited by Tuomi and Sarajärvi, 2009). Data reduction is shown in the table below;

Table 5. Example showing data reduction

Original expression	Reduced/simplified expression
“They gave good examples how it had happened to others and how had they explained their eating disorder, it encouraged me.”	Explaining examples
“About future, it was discussed with my own nurse what I am going to do and what would I like in future?”	Discussion about future
“The doctor was such a person, who let me think about other issues than sickness.”	Thoughts about sickness gone

Table 6. Data reduction in my research work

Original expression	Reduced/simplified expression
An antioxidant is a substance that delays the onset of oxidation or slows down the rate at which oxidation occurs	Definition of antioxidant
Many antioxidants are reducing agents that are readily oxidized bimolecular oxygen or reactive free-radical intermediates, effectively terminating chain reactions and inhibiting other oxidation processes	Antioxidant activity/ Mechanism of antioxidant reaction
Ascorbic acid is a powerful antioxidant and reducing agent capable of donating one or two electrons in biochemical redox reaction.	Example of antioxidant and its reaction
Along with other antioxidants, ascorbic acid is commonly used as additive E300 for preventing oxidative rancidity.	Applications of antioxidants
In living organisms, carotenes and other group A vitamins act as antioxidants, protecting the cells from UV light, peroxides, and free radicals, including a highly reactive “singlet oxygen” produced by photosynthesis.	Antioxidant activity

5.2.3 Need to select data management process

Qualitative research done by inexperienced or lone-wolf researchers are vulnerable while starting data management. Kvale (1988) provides a wry analysis of the naive question, “How shall I find a method to analyse the 1,000 pages of interview transcripts I have collected?” His first answer is, “Never conduct interview research in such a way that you arrive in a situation where you (have to) ask such a question.” (cited by Miles and Huberman, 1994, p 429).

Qualitative data refers to essence of people, objects and situation (Berg,1989).

Essentially raw experience takes form of words and extended text, still or moving images.

Processing of data collection can be time consuming before data analysis. Werner and Schoepfle (1987b) suggest a system for data storage and retrieval prior to actual data collection and emphasize the importance of a clear indexing system. (cited by Miles and Huberman, 1994, p 430).

5.2.4 General steps involved in data management process

Levine (1985) proposed five general functions regarding storage and retrieval (cited by Miles and Huberman, 1994, p 430).

- (a) Formatting (how materials are laid out, physically embodied and structured into types of files).
- (b) Cross-referral (linkage across different files)
- (c) Indexing (defining codes, organizing them into structure, and pairing codes with specific parts of the database).
- (d) Abstracting (condensed summaries of documents or extended field notes)
- (e) Pagination (numbers and letters locating specific material in field notes)

5.2.5 Process of inductive data analysis as presented by Miles and Huberman

Miles and Huberman (1994) suggest that inductive data analysis is a 3-step process.

- 1) Data reduction or simplification
- 2) Data categorization
- 3) Abstraction

In Fig. 5 below, the stepwise process of data analysis is presented (Tuomi and Sarajarvi, 2009, p 108-9).

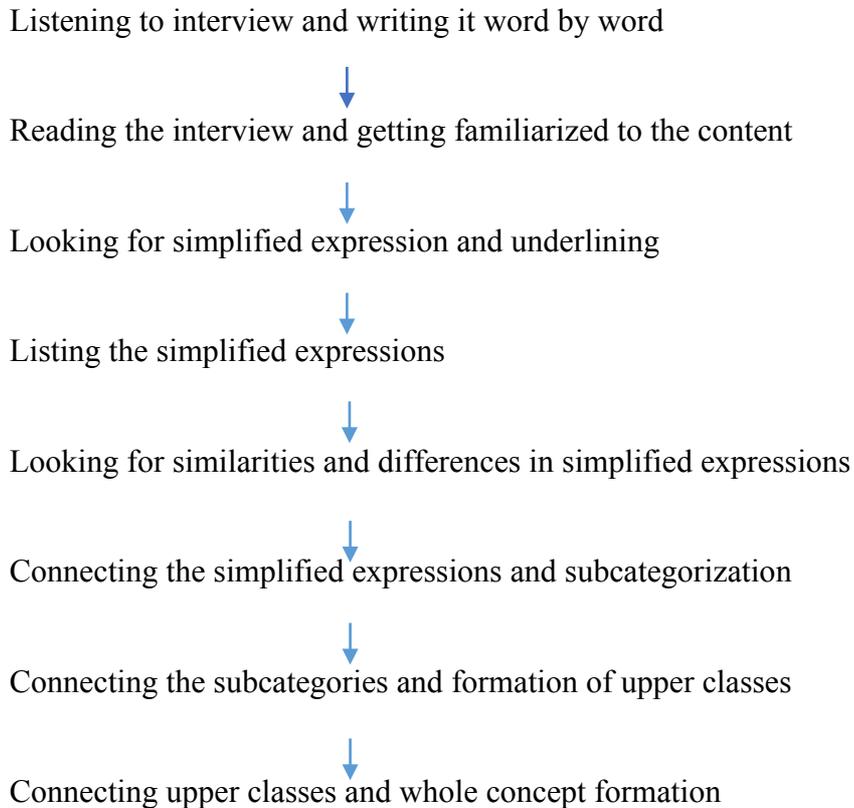


Figure 5; Process of content analysis of data

5.3 Stepwise process of inductive content analysis carried out in this research work

5.3.1 Preparation phase

This step is common to both inductive and deductive approach, in which many words of the text are classified into much smaller content categories.

The first step in preparation phase is to select the unit of analysis which can be a word or a theme. Before selecting the unit of analysis, it should be decided what is to be analysed and in what detail. Unit of analysis can also be a letter, word, sentence, portion of pages or words, the number of participants in discussion or time used for discussion. The written material is read through several times to get immersed in the data and get familiarized with it (cited by Elo and Kyngäs, 2008).

Method used in this research work;

Before selecting the unit of analysis, it was discussed with the supervisor and decided what information related to antioxidants had to be analyzed in the curriculum books and if there would be no description about the topic in upper secondary school course books, then what other information related to the topic could be analyzed and in what detail. The unit of analysis had to be selected right in the first step which came out to be antioxidants.

5.3.2 Data collection

About content categorization in Finnish research standard practice, Pietilä (1973) and Eskola (1975) refer to Bernard Berelsoni's definition presented in 1952:

“Sisällön erittely (content analysis) on kommunikaation ilmissisällön objektiivista, systemaattista ja määrällistä kuvailua varten soveltuva tutkimustekniikka.” (cited by Tuomi and Sarajärvi, 2009, p 105).

It is interesting to note that in many resources, content analysis and content categorization are taken to be synonyms.

Content categorization is quantitative picture of contents of the text while content analysis is the word analysis of the content of the document. This limitation does not let us speak about quantification of data as an analytical method while talking about content analysis (Tuomi and Sarajärvi, 2009, p 106). He further mentions about the difference as,

“Sisällönanalyysi kertoi siis huomattavasti enemmän ja antoi analysoiduista pro gradu-töistä ja tutkimuksista tarkempaa tietoa kuin sisällön erittely.”

Method used in this research work;

For obtaining high-quality data, access to Finnish curriculum books and international curriculum books was made possible with the help of teachers from the university school, Turun normaali koulu and book publishers, Sanomapro had graciously sent the paper version of the books for helping this analysis and Otava had kindly given access to digital books for a limited time.

All the books were studied thoroughly to check if the multidisciplinary topic regarding antioxidants has been sufficiently described in the curriculum meeting the requirement of relevance and connecting chemistry with biology and health education. Inductive approach involves specific to general approach. Firstly, the main topic antioxidants had to be searched and then further categorized by using its examples, mechanisms and examples were further sub-categorized. Data collection was the very

first step including all the information related to main heading, categories and then sub-categories. It took long to process the data obtained from different books used in national and international curriculum. Data was formatted into types of files, cross-referred and indexed. Finally, the condensed summaries of the documents had to be developed as part of abstraction step.

5.3.3 Formulation of categories while organizing qualitative data

After preparation, next step is organizing qualitative data which includes open coding, creating categories and abstraction. Open coding is to write notes and headings written in the text while reading it. The written material is read through again and again, and as many headings as necessary are written down in the margins to describe all aspects of the content (Hsieh and Shannon, 2005). The headings are transferred to the coding sheets and categories generated. Categories are created to describe the phenomenon with better understanding. Formulation of categories helps the researcher to decide, as to which things to put in the same category.

Method used in this research work

Reading the books thoroughly, it came to knowledge that antioxidants were rarely mentioned as antioxidants in the national curriculum books, rather their examples had been described with their sources and their functions. IB Chemistry book had mentioned about antioxidants along with the definition, different types of antioxidants and their function as well as mechanism of reaction, hence it was easy to display information but in the biology and health education books, the term “antioxidants” was mentioned in the disguise of vitamins mostly, so the content was categorized forming classes looking for similarities and differences accordingly to get better understanding. It made the task easier, as to which things to put in the same category. Simplified expressions were connected and subcategories were formed which led to formation of upper classes and finally the whole concept formation. (Miles and Huberman,1994)All the results of the content analysis carried out had to be then documented as an organized and compressed assembly of all the information presented in IB chemistry and biology books as well as Finnish course books used in the upper secondary school related to chemistry, biology and health education. This part of data analysis is data display (cited by Miles and Huberman,1994, p 428).

Data was categorized into types of antioxidants mentioned in the course books. IB chemistry had directly mentioned about antioxidants along with the definition,

different types of antioxidants and their function as well as mechanism of reaction, hence it was easy to display information but in the biology and health education books, the term “antioxidants” was mentioned in the disguise of vitamins mostly, so the content was categorized forming classes to carry out the word analysis of the content of the document (Tuomi and Sarajärvi, 2009, p 106). It helped to decide which information needs to be in the same category and thus understand it better.

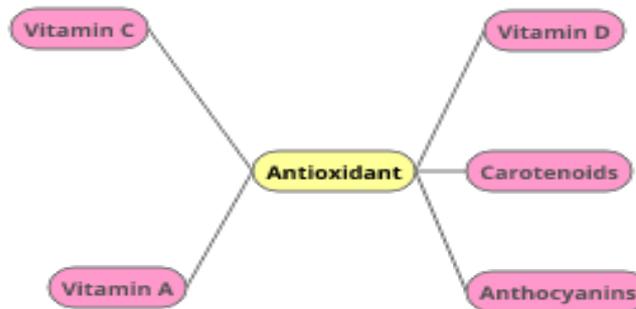


Figure 6. Antioxidants

5.3.4 Abstraction

Abstraction means to formulating a general description of the research topic through generating categories. Each category is then named using content-characteristic words. Sub-categories with similar events and incidents are grouped together as categories and categories are grouped as main categories. The abstraction process continues as far as possible and reasonable. (Elo and Kyngäs, 2008).

Hämäläinen(1987)kuvaabstahointia prosessiksi,jossa tutkija muodostaa yleiskäsitteiden avulla kuvauksen tutkimuskohteesta.Tällöin teoriaa ja johtopäätöksiä verrataan koko ajan alkuperäisaineistoon uutta teoriaa muodostettaessa. Abstrahoinnissa empiirinen aineisto liitetään teoreettisiin käsitteisiin ja tuloksissa esitetään empiirisestä aineistosta muodostettu malli, käsitejärjestelmä, käsitteet tai aineistoa kuvaavat teemat. Tuloksissa kuvataan myös luokittelujen pohjalta muodostetut käsitteet tai kategoriat ja niiden sisällöt. Johtopäätösten tekemisessä tutkija pyrkii ymmärtämään, mitä asiat tutkittaville merkitsevät. Tutkija pyrkii siis ymmärtämään tutkittavia heidän omasta näkökulmastaan analyysin kaikissa vaiheissa (cited by Tuomi and Sarajärvi, 2009, p 112).

Method used in this research work;

Antioxidants were mostly described as vitamins, carotenoids or anthocyanins in different course books, with the exception that chemistry IB book directly addressed the topic of antioxidants. Abstraction, for example formulating a general description of the research topic through generating categories was to be carried out. The categories generated were, therefore, vitamins, carotenoids and anthocyanins.

After reading the material again and again and getting familiarized to the content, these classes were underlined, listed and all the information content related to these classes was then subcategorized. Subcategories with similar events and incidents were grouped together as structure, function, examples, sources, applications and reactions and pagination was also carried out as part of analysis to specify the numbers and letters locating specific material in the research content.

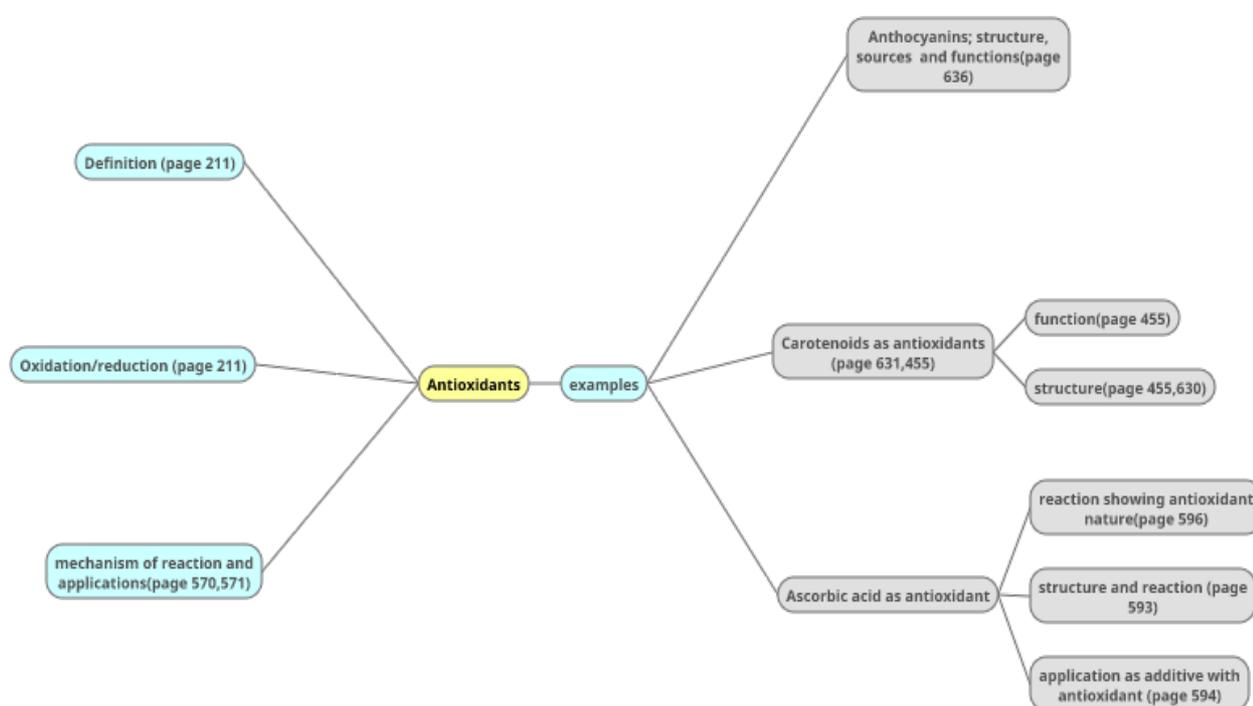


Figure 7. Process of abstraction showing categorisation and sub-categorisation

6 Results of the content analysis of the course books

The main objective of this book review was to conduct qualitative analysis of the theory about antioxidants discussed in Finnish curriculum books and international curriculum books.

6.1 Content analysis of course books used in international curriculum

Five course books used in international curriculum had been studied including one chemistry and four biology books while study books for chemistry and biology, one for each, had also been studied to analyse the information regarding free radicals and antioxidants.

6.1.1 IB Chemistry book and study guide

IB Chemistry had the most information and the word antioxidant had been mentioned fairly well emphasizing the importance of this concept, which is really needed to be taught to this age group level. (see Fig 6, p 30 and Fig 7, p 31)

In the IB Chemistry book, Oxford IB diploma programme 2014 Edition, Brian Murphy, Gary Horner, David Tarcy and Sergey Bylikin define antioxidants as;

“An antioxidant is a substance that delays the onset of oxidation or slows down the rate at which oxidation occurs.”

They also use the mnemonic OILRIG for remembering oxidation as loss of electron and reduction as gain of electron. (Murphy et al, 2014, p 211)

According to them, oxidative rancidity can be avoided by adding natural or synthetic antioxidants including vitamins A, C and E. (Murphy et al, 2014, p 570)

Brian Murphy and others conclude that, *“Many antioxidants are reducing agents that are really oxidized by molecular oxygen or reactive free-radical intermediates, effectively terminating chain reactions and inhibiting other oxidation processes.”* (Murphy et al, 2014, p 571)

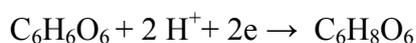
Regarding structure of carotenes, Brian and others agree to the fact that efficient antioxidant nature is due to the electron conjugation and they react readily with molecular oxygen and free radicals. (Murphy et al, 2014, p 592)

Redox reaction of Vitamin C is shown with structural and molecular formulae representing its antioxidant nature. (Murphy et al, 2014, p 593-4)

Ascorbic acid is a powerful antioxidant and reducing agent capable of donating one or two electrons in biochemical redox reactions, for example:



The oxidized form of vitamin C, dehydroascorbic acid, can be reduced to ascorbic acid by certain enzymes or glutathione.



Murphy and others describe carotenes as antioxidants (Murphy et al., 2014, p. 631) and sensitive to photo-oxidation.

“In living organisms, carotenes and other group A vitamins act as antioxidants, protecting the cells from UV light, peroxides, and free radicals, including a highly reactive “singlet oxygen” produced by photosynthesis.”

Study guide for IB chemistry not only included the brief theory about vitamins but also the mechanism of free radical reaction involving initiation, propagation and termination steps.

6.1.2 IB biology books

IB Biology books have mentioned about this theory in the Diet option under the heading of vitamins especially vitamin C or ascorbic acid is discussed as an antioxidant.

IB Oxford Biology Edition 2014(D-option) Human Physiology

Andrew Allott and David Mindorff have defined vitamins as,

“Vitamins are organic compounds that are needed in very small amounts because they cannot be synthesized by the body but must be obtained from the diet. They serve a variety of roles such as co-factors for enzymes, anti-oxidants and hormones. The word vitamin is derived from the words “vital amine” as the first vitamins to be discovered contained an amino group. Other vitamins discovered since do not necessarily contain amino group such as vitamins A, C, D and E.”

Allott has described vitamins as antioxidant (Allott and Mindorff, 2014, p 663) along with sources and structure of other vitamins and has mentioned in detail how the free radicals of tobacco smoke (Allott and Mindorff, 2014, p 705) disturb the function of the lungs and damage the lung tissues as a result of their oxidation reactions, causing emphysema.

Extract of information from D option; Human Physiology as discussed in IB Oxford biology edition 2014 is as follows;

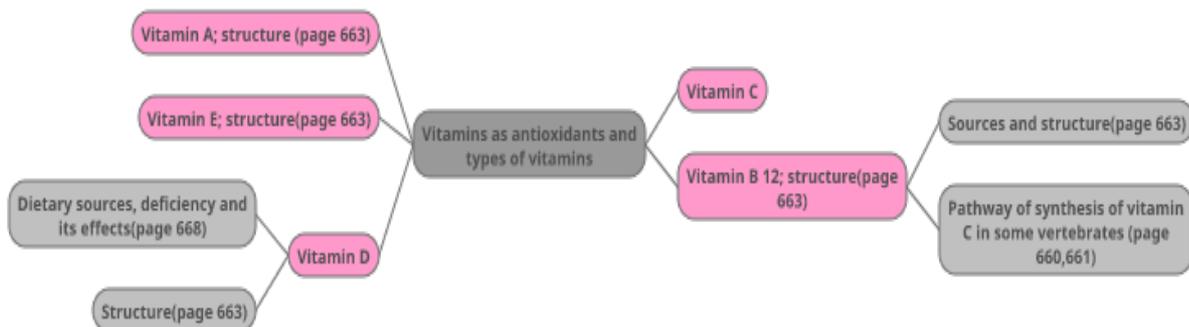


Figure 8. Extraction of information from Oxford IB biology

Oxford study guide for IB biology

Oxford study guide also gives definition and examples of vitamins (Allott, 2014, p110) and their oxidation and reduction reaction mechanism (Allott, 2014, p 73).

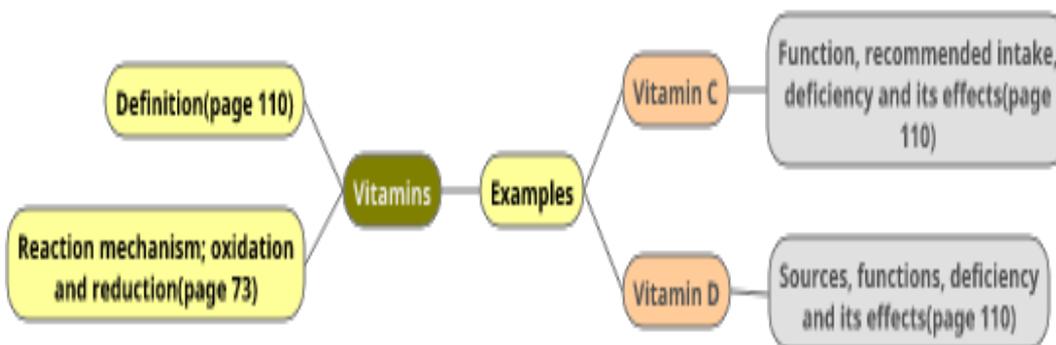


Figure 9. Extraction of information from Oxford study guide for IB biology

IB Hodder biology

In Hodder biology as well, Chris Clegg has clearly mentioned vitamin C as an antioxidant (Clegg, 2010, p 2) in the option Diet and explained mechanism of oxidation and reduction (Clegg, 2010, pp 270-1, 446)

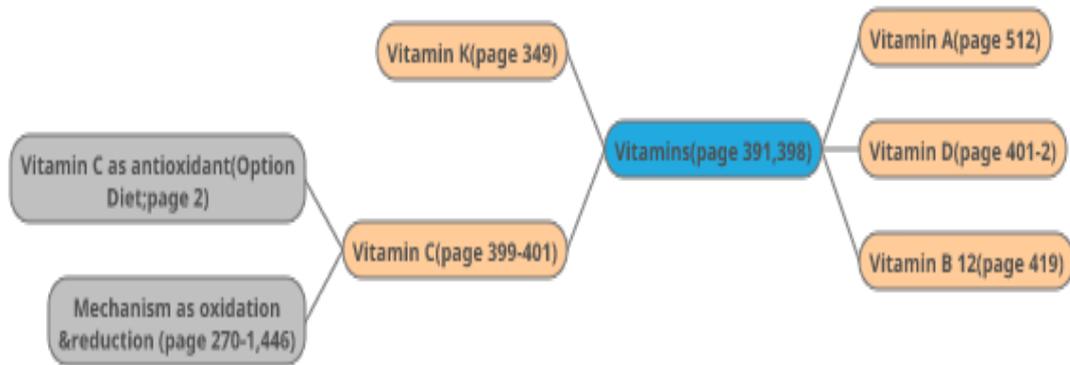


Figure 10. Data collected from IB Hodder biology

C. J. Clegg in Hodder biology, option D with the heading human physiology, describes this fact as an essential idea, “A balance diet is essential to human health”. In chapter 15, table 15.3, detailed classification of vitamins in human diet is illustrated along with the sources and functions of all vitamins. (Clegg, 2010, p 2)

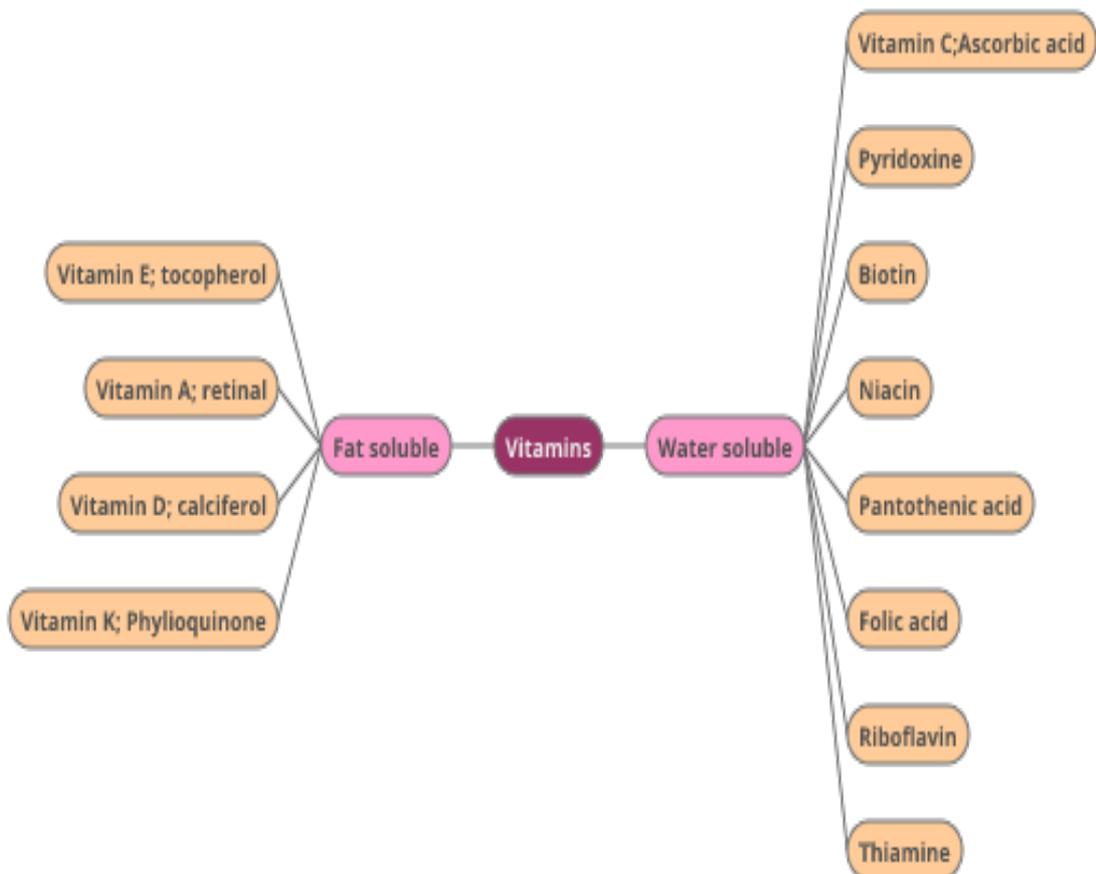


Figure 11. Detailed classification of vitamins given in IB Hodder biology

In Hodder Biology, C.J.Clegg agrees to the fact that, “Ascorbate is also a radical-trapping antioxidant”, while describing the sources, functions and signs of

deficiency of vitamin C.(page 9).He gives the historic details of discovery of vitamin C including experimentation on guinea pigs and later with human volunteers(1942-1944)(Clegg, 2010, p. 10).Required intake, function, effects of deficiency of vitamin D are also explained in parallel to vitamin C(Clegg, 2010, p 11).

IB Cambridge biology

Cambridge Biology, second edition, shows an easy way to remember oxidation and reduction by thinking of the words OIL RIG. The book shows with diagram and table (Walpole et al,2014, p 268) how oxidation and reduction are linked processes in a biochemical reaction.

Table 7. Changes involved in oxidation and reduction (Walpole et al.,2014,p.268)

Oxidation	Reduction
Loss of electrons	Gain of electrons
Loss of hydrogen	Gain of hydrogen
Gain of Oxygen	Loss of Oxygen

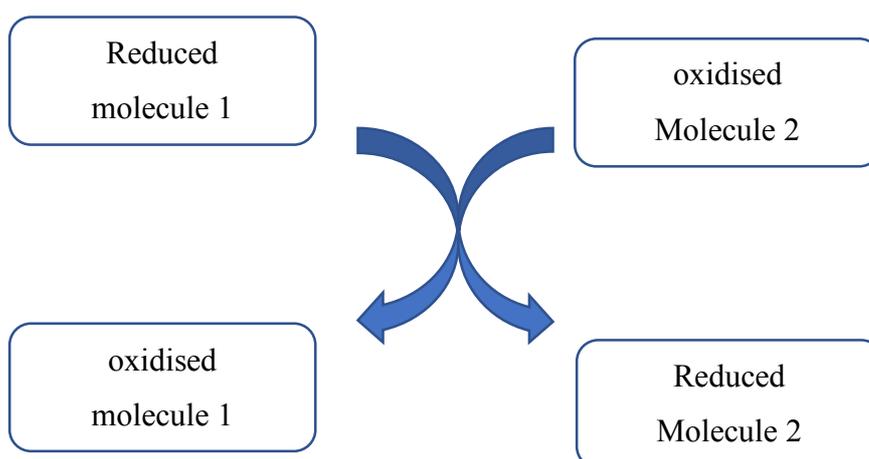


Figure 12.Oxidation and reduction are linked processes

Analysis of Cambridge biology (Walpole et al, 2014) reveals the following data related to antioxidants.



Figure 13. Data collected from Cambridge biology

Cambridge Biology includes vitamins C and D in Option D (accessed electronically), Human health and physiology but doesn't mention about antioxidant nature of vitamin C.

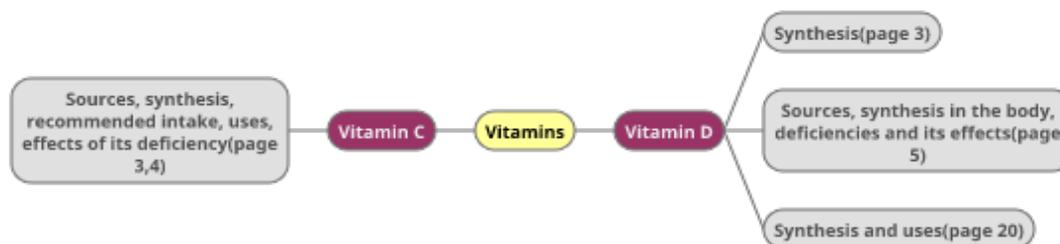


Figure 14. Vitamins in Option D of Cambridge biology

6.2 Content analysis of course books used in Finnish curriculum

A request by mail had been sent to the publishing companies Otava and SanomaPro to kindly provide the books used by upper secondary school students for this book review. SanomaPro had some queries about how these books were going to be used which are all written in Finnish language. Getting the explanation, SanomaPro had very kindly sent the course books related to chemistry, biology and health education subjects in upper secondary schools in Finland. Bios 4 and Bios 5 were not yet available and same was with Terve 3, as these were going to be published later.

6.2.1 TERVE 1

Terve 1, Terveiden perusteet (Reinikkala et al, 2016) had described the vitamins as antioxidants and theory extracted from that book is as under;

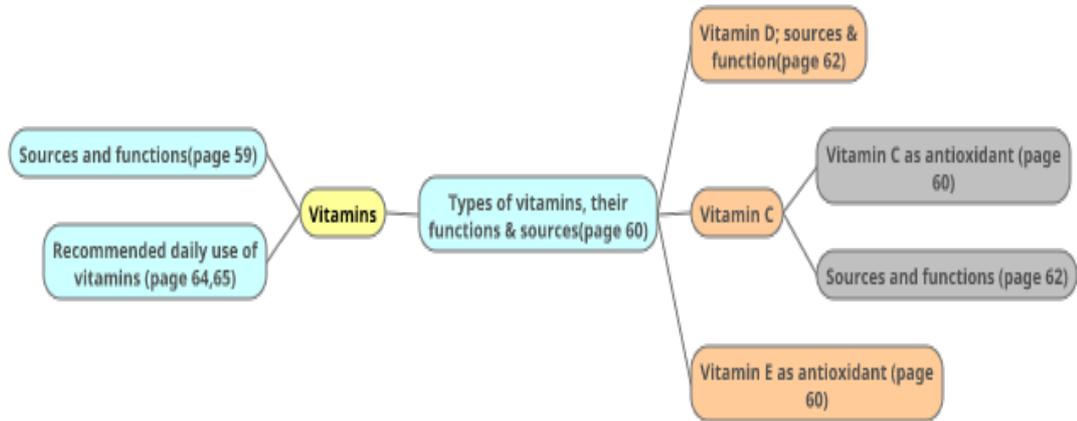


Figure 15. Data extracted from Terve 1

6.2.2 TERVE 2

No information related to antioxidants was found in Terve 2, Ihminen, ympäristö ja terveys (Reinikkala et al, 2016).

6.2.3 KEM 1, KEM 2 and KEM 3

In Kem 1, Kemiaa kaikkialla (Koskinen, 2016) nothing related to structure, sources or functions of antioxidants was found and not even about vitamins. In Kem 2, ihmisen ja elinympäristön kemiaa (Koskinen, 2016), it had been mentioned about the structure and function of Vitamin A and β -carotenoids on page number 79 (Koskinen, 2016, p 79) while the mechanism of oxidation and oxidation was only found in Kem 3, reaktiot ja energia (Koskinen, 2016, p 90).

6.2.4 BIOS 1, BIOS 2 and BIOS 3

Bios 1, Elämä ja evoluutio, and Bios 2, ekologia ja ympäristö (Happonen et al, 2016), had neither information about vitamins, nor about antioxidants. In Bios 3, solu ja perinnöllisyys (Happonen et al, 2016), the function of antioxidants has been explained with the difference between oxidation and reduction as, “Hapetus-pelkistysreaktioissa hapettuvan aineen atomit luovuttavat elektroneja pelkistyvän aineen atomeille. Hapettuminen ja pelkistyminen tapahtuvat aina samanaikaisesti: kun jokin aine hapettuu, toinen pelkistyy.”

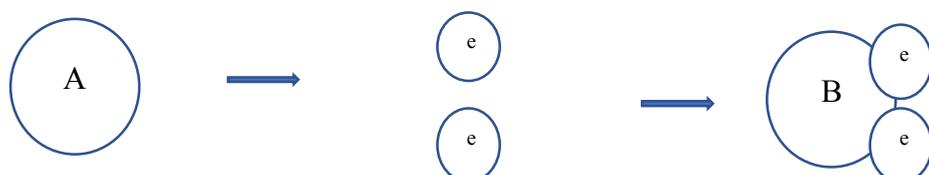


Figure 16. Reactions involved in activity of antioxidants as shown in BIOS 3, solu ja perinnöllisyys

Bios 3 describes carotenoids as, “kasvisolujen väriaineet” and doesn't say anything about their antioxidant nature.

6.2.5 Koralli 1,2 and 3

Otava books for Biology include Koralli 1,2 and 3(electronically accessed). No information regarding anti-oxidants, free radicals and nothing about vitamins was found in these books.

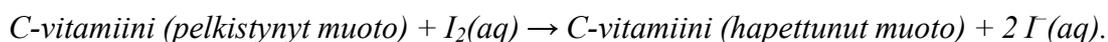
6.2.6 Mooli 1 (experimental work)

Otava chemistry book, Mooli 1, Kemiaa kaikkialla (electronically accessed), has well designed laboratory activity with the heading, tuoremehun C-vitamiinipitoisuus, which includes the experimental work to determine the amount of vitamin C in fresh juice. It not only says about the antioxidant nature of vitamin C but shows in detail the oxidation and reduction reactions of vitamin C.

C-vitamiini on vesiliukoinen vitamiini, joka toimii elimistössä hapettumisen estoaineena eli antioksidanttina. Tämä tarkoittaa, että C-vitamiini hapettuu helposti, mikä suojaa muita yhdisteitä hapettumiselta. C-vitamiinilla on tärkeä merkitys myös elimistössämme eniten esiintyvän proteiinin, kollageenin, valmistumisessa.

In addition to daily intake need, oxidation and reduction of vitamin C is well discussed, as

Mehun C-vitamiinipitoisuuden tutkiminen perustuu reaktioon, jossa C-vitamiinia sisältävän liuoksen annetaan reagoida alkuainejodin kanssa. Tässä reaktiossa C-vitamiini hapettuu ja alkuainejodi pelkistyy jodidi-ioneiksi. Tätä reaktiota voidaan kuvata seuraavasti:



6.2.7 Mooli 2 (experimental work)

Mooli 2, Ihmisen ja elinympäristön kemiaa (electronically accessed), in chapter 1(mooli, ainemäärä ja konsentraatio) only describes determination of concentration of vitamin C in fresh juice using molar mass and number of moles but doesn't say anything about its anti-oxidant nature.

6.2.8 Tarmo 1 and 2

Otava books for health education include Tarmo 1 and Tarmo 2. Tarmo 2 (electronically accessed), didn't include any theory about vitamins but Tarmo 1 includes chapter 3 with the main heading as Ravitseemus with all the details of types of vitamins.



Figure 17. Data extracted from Tarmo 1

In chapter 4, with the main heading of Erityisruokavaliot ja painonhallinta, it says about antioxidants as,

“Vegaaniruokavalion riskejä ovat B12-vitamiinin, D-vitamiinin, kalsiumin ja jodin niukka saanti. Myös raudan saanti voi olla vähäistä, koska se imeytyy kasvikkunnan tuotteista heikommin kuin eläinkunnan tuotteista. Vegaaniruoka sisältää runsaasti antioksidantteja, niukasti tyydyttyneitä rasvoja ja kolesterolia, mikä edistää terveyttä.”

7 Research method for case study used for experimental work

7.1 Definition

Merriam-Webster's dictionary (2009) defines a case study (cited by Flyvbjerg, 2011, p 301) as follows;

Case study is an intensive analysis of an individual unit (as a person or community) stressing developmental factors in relation to environment.

This definition shows focus on an “individual unit”, which may be studied qualitatively or quantitatively, analytically or hermeneutically, or by mixed methods. Secondly, definition shows that case studies are “intensive”, thus comprising of more detail, richness, completeness and variance. Thirdly, case studies stress “developmental factors”, and finally they focus on “relation to environment”, that is, context. (cited by Flyvbjerg, 2011)

7.2 Strengths and weaknesses of the method

The strengths and weaknesses of this research method are given as follows;

Table 8. Case study with its strengths and weaknesses

Strengths	Weaknesses
<ul style="list-style-type: none">• Depth• High conceptual validity• Understanding of context and process• Understanding of what causes a phenomenon, linking causes and outcomes• Fostering new hypotheses and new research questions	<ul style="list-style-type: none">• Selection bias may overstate or understate relationships• Weak understanding of occurrence in population of phenomena under study• Statistical significance often unknown or unclear

Case study is not a methodological choice, but a choice of object to be studied. A case is chosen for study and it could be studied in many ways. Cases are studied and recorded in many professions as a physician studies the sick child. The child's symptoms are both qualitative and quantitative but the physician's record is more quantitative. A social worker studies a neglected child whose symptoms of neglect are both qualitative and quantitative but the social worker's record is more qualitative. Hence case study is defined by interest in individual cases, not by the inquiry methods used in study. (Stake, 1994, p 236)

7.3 Types of case study

Three types of studies are identified based on purposes for studying the case. (Stake, R.E, 1994, p 237)

Intrinsic case study;

This study is carried out for better understanding of a particular case primarily because of the intrinsic interest in it. e.g. a particular child, conference or curriculum.

Instrumental case study;

A particular case is examined in depth, to provide insight into an issue or refinement of theory. The case is of secondary interest playing a supportive role to facilitate understanding of something else.

Collective case study;

The joint study of a number of cases carried out into the phenomenon, population or general condition. The case itself is of even less interest. Individual cases in the study may be similar or dissimilar, understanding of which, will lead to better understanding of still large collection of cases.

7.4 Data required for case study

While studying a case, following data needs to be gathered for a particular case. (Stake ,1994, p 238):

- 1) the nature of the case
- 2) its historical background
- 3) the physical setting
- 4) other contexts including economic, political, legal and aesthetic
- 5) other cases through which this case is recognized
- 6) those informants through whom the case can be known

8 Results of the case study conducted in present research work

In the case study during the research work, the questionnaires were prepared for both visiting groups of students. The questions were added according to the level of knowledge of the students and level of information presented to the students in the introduction to the topic of antioxidants and free radicals. All the students had to express the learning outcomes in form of the information asked in the questionnaires.

8.1 Data regarding age and sex of the participating students

Group of grade 9 students included 20 students ranging between the age of 14-16 years old ones. Among the female students, one female student was 14 years old and one was 16 years old while eight other female students were 15 years old. Among the male

students, one was 16 years old, two students were 14 years old while four of them were 15 years old. Three of the students didn't mention about it (see graph, fig 18).

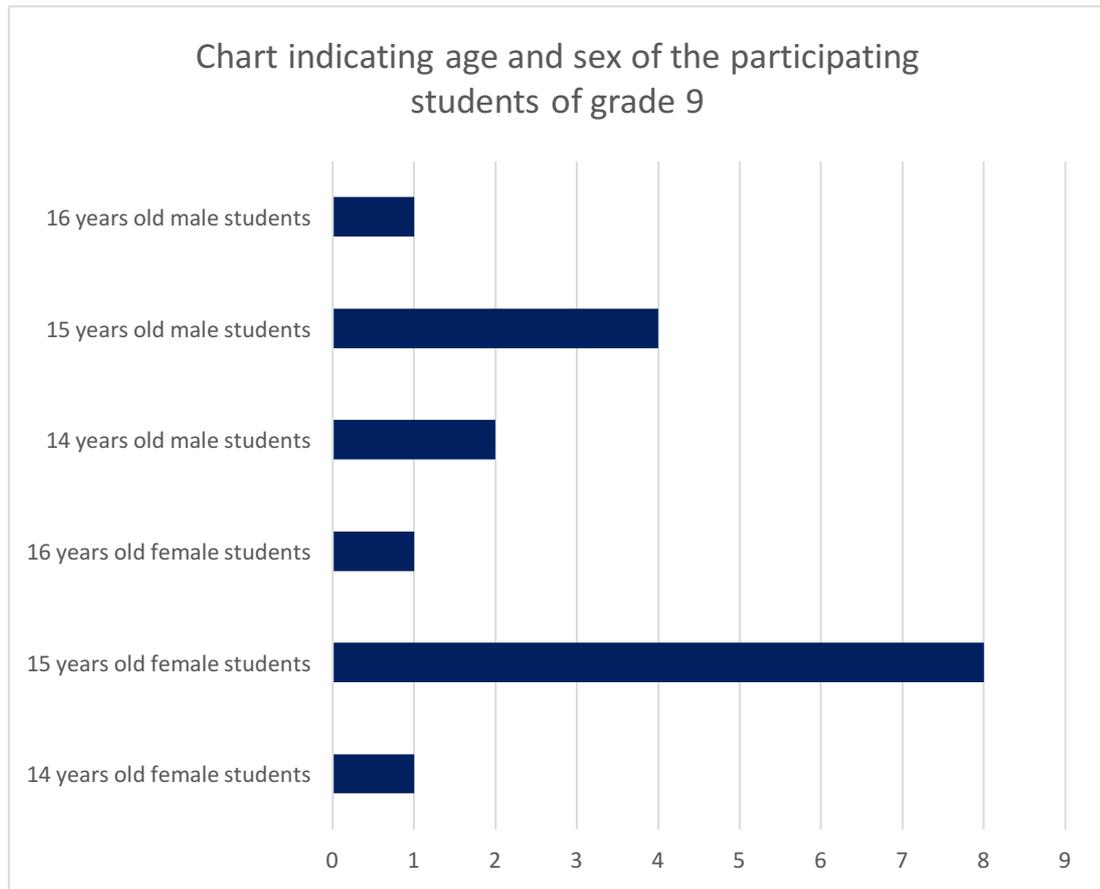


Figure 18. Data regarding age and sex of the participating students from grade 9

From Pre-IB group of students, only 22 students filled in the questionnaires. Among them, eighteen female students were 16 years old, one male student was 15 years old while one male student was 16 years old. Two students didn't mention about their sex. (see graph, fig. 19)

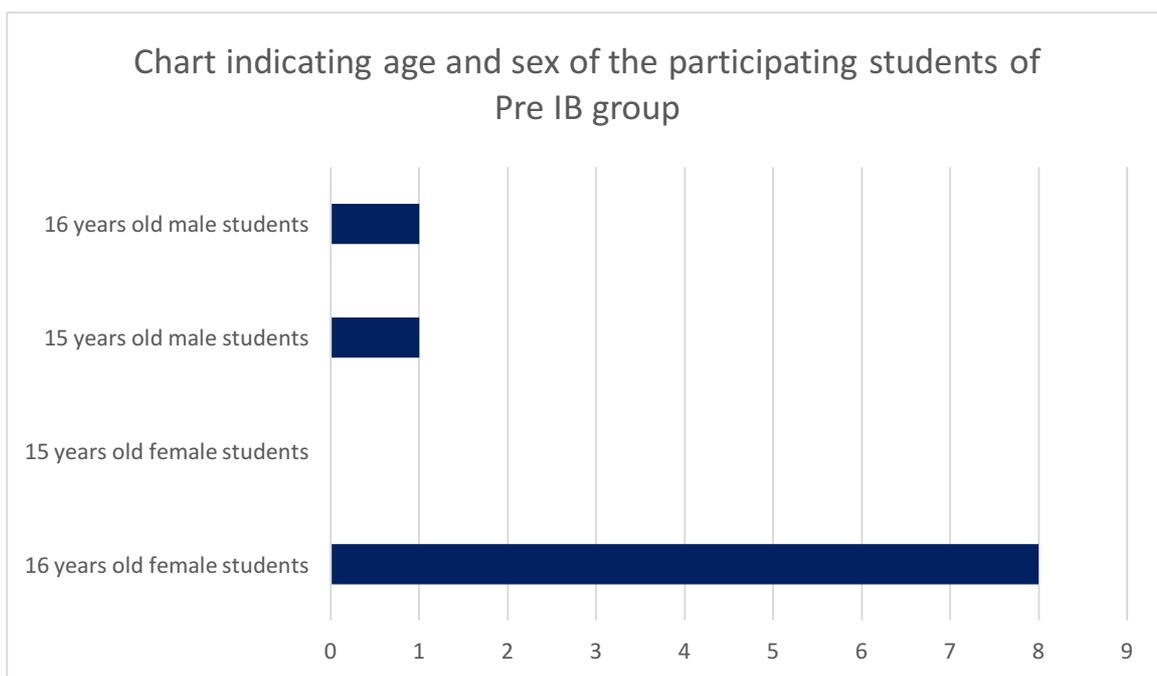


Figure 19. Data regarding age and sex of the participating students from Pre-IB group

8.1.1 Data collected from the questionnaires

Questionnaires from grade 9

The outcome of the data collection from the questionnaires filled out by the students of grade 9 is displayed in the table and graphs below;

Table 9. Results of data collected from students of grade 9

	Strongly disagree	disagree	agree	Strongly agree
Introduction to antioxidants was interesting		1	12	3
Experimental work was interesting			8	9
Instructions were easy to understand		3	9	5
I understood theory of experimental work			13	4
I learnt new chemistry technique		1	5	11
Visit to chemistry OpiLUMA lab increased my interest in chemistry subject		2	10	4
I would like to visit OpiLUMA lab again		4	7	6
I would like to know more about healthy food and antioxidants		4	9	4

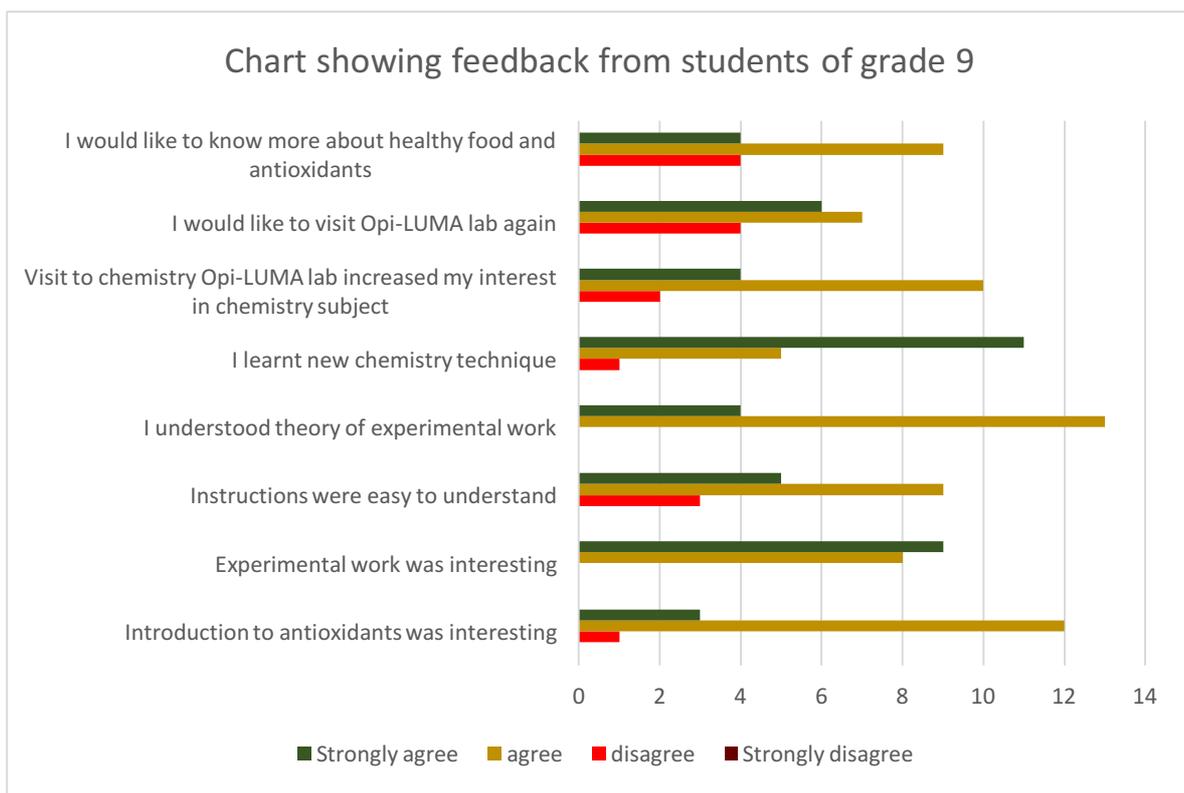


Figure 20. Graph showing outcome of the questionnaires from grade 9 students

Questionnaires from pre IB

The outcome of data collection from the questionnaires filled out by the students of Pre IB group is shown in the table and graphs below;

Table 10. Results of data collected from students of Pre-IB group

	Strongly disagree	disagree	agree	Strongly agree
Introduction to antioxidants was interesting			20	1
Introduction to adulteration test was interesting		3	17	
Experimental work was interesting			10	11
Instructions were easy to understand		6	12	3
I understood theory of experimental work		4	17	
I learnt new chemistry technique			11	10
I would like to visit OpiLUMA lab again		2	9	10
I would like to know more about healthy food and antioxidants		4	10	6
Visit to chemistry department increased my interest in chemistry subject		2	10	8

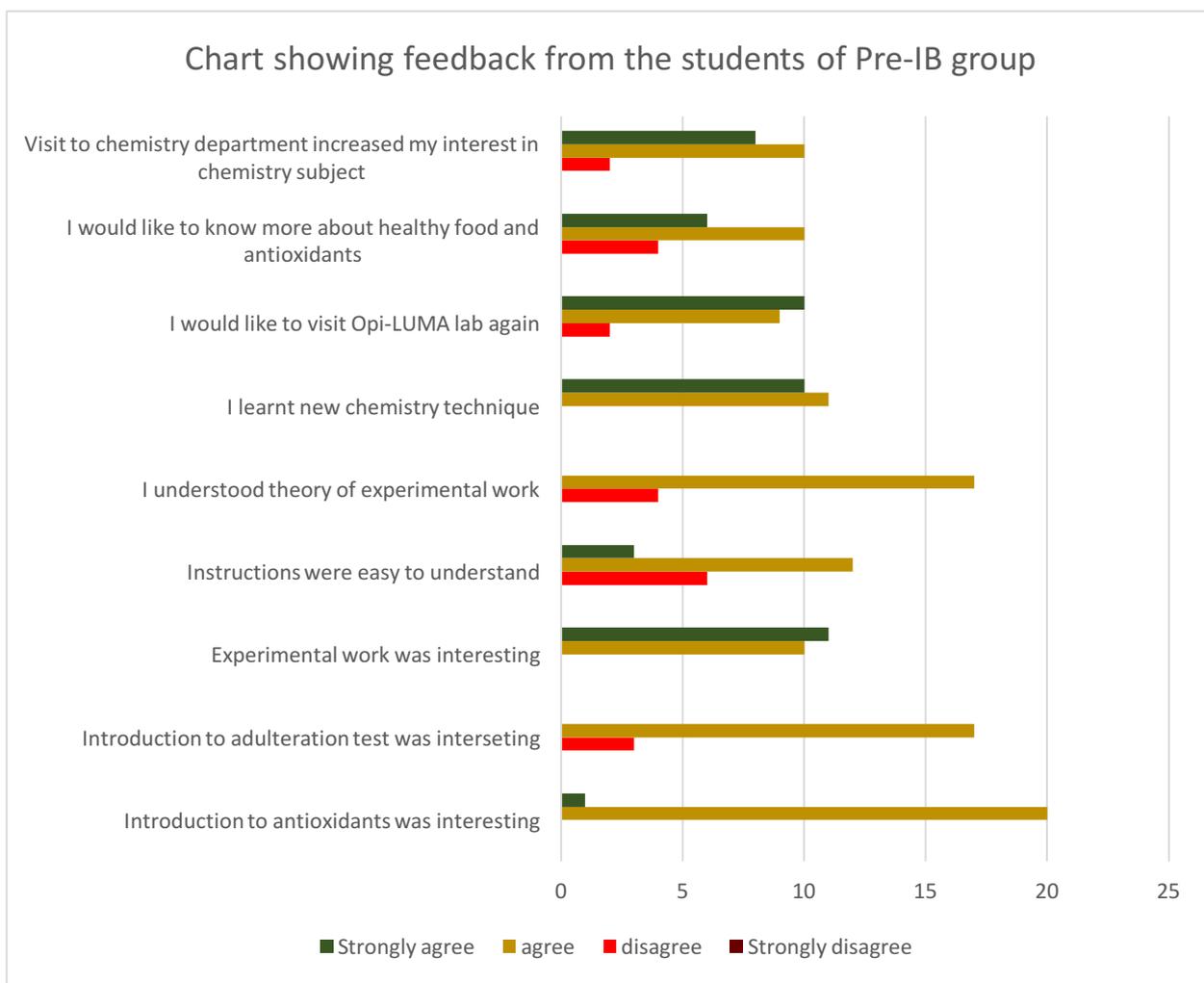


Figure 21. Outcome of the data collected from questionnaires filled by the students of Pre-IB group

8.1.2 Data regarding the most interesting part of the planned experimental work

As part of questionnaire, students were given the opportunity to tell which part of the experiment was more interesting. The answers by the students reflected their most interest towards magnetic stirring, colour changes in Briggs and Rauscher reaction and using new kind of laboratory equipment as micropipettes. Some students expressed, moreover, their wish to know in detail how the antioxidants work and their function in the body.

One of the students had surprisingly provided connection of the topic with the relevance of chemistry to everyday life. He said, “I would like to know more about antioxidants in order to provide healthy food to my customers when I establish my own business in form of a restaurant”.

8.1.3 Feedback by the students regarding experimental work

Students had been given the opportunity to express themselves freely if there was something else worth mentioning, in addition to the questions in the questionnaire.

“I liked to do everything we did.”

“I came to know about the sources of antioxidants”.

“I would like to know more about antioxidants and the theory behind it in more depth how and why the changes occurred.”

“The experiment was interesting but I really didn’t understand the theory behind it. I would like to understand the chemistry behind it.”

“The whole thing was pretty interesting”.

“I would like to know the chemistry involved in series of colour changes.”

“I would like to measure the exact quantity of antioxidants in food through experimental work.”

“I don’t know exactly what I would like to know more about this topic but I do know it that I want to know it more in general. Chemistry is fascinating.”

One of the students clearly reflected her understanding of the topic of antioxidants by writing, *“The more time it takes from blue to colourless, it contains more antioxidants”.*

9 Discussion;

Antioxidants are highly important chemical compounds regarding their defensive role to sustain our health and they should have been considered as an essential part of our daily diet. It is highly needed to give awareness to the students of upper secondary school about the chemistry of antioxidants and their function in our body, thus emphasizing the concept of eating balanced diet.

9.1 Experimental work designed for the research

Taking into consideration the requirement of student-centered activities and inquiry-based approach in the educational system independent of level of development through student laboratory work and co-operative learning methods, by modern pedagogies of chemistry learning, the experimental work was designed irrespective of their individual learning outcomes.

To enhance teacher-scientist interaction to chemistry teachers’ education (Vesterinen and Aksela, 2009), study design was planned based on OpiLUMA laboratory activities

for the secondary school students and later qualitative content analysis of the curriculum books used in the upper secondary school system.

9.2 Scientific literacy for all

This study design is rightly connected to the aim of “scientific literacy for all” (PISA) making students understand the objectives of the chemistry curriculum (Eilks et al, 2013). It clearly defines the relevance of chemistry to the students by satisfying their curiosity and explaining the interconnection between different branches of life sciences as well as interdependence and interaction of science and human health, as the topic of antioxidants is multidisciplinary presenting well the connection between chemistry, biology and health science.

To reduce the difficulties in understanding, it was decided to stay mainly to the macro level of activities in chemistry, the descriptive level as suggested by Johnstone, describing the concept of free radicals and antioxidants and then gently introduce the microscopic level by interpreting the specific biological role of antioxidants in human health to prevent damage by the free radicals, with the help of pictures and power point.

Definition, sources, examples, important physiological role of free radicals and antioxidants in human health are discussed on this descriptive level. Introduction to theory of Briggs-Rauscher reaction to determine level of antioxidants in food samples and inhibition time measurement in the designed laboratory technique for the secondary school students was just limited to the descriptive level of chemistry learning. This approach was found to be highly effective without burdening the working memory capacity of the students. (Eilks et al, 2013).

This approach is exactly in accordance with the strategy of moving from macroscopic to molecular level, i.e. from big to small. Symbolic level to represent chemical equations with formulae showing all the reaction mechanism of antioxidants involving oxidation and reduction reactions was removed altogether from the study design to avoid perception of chemistry as an abstract and difficult topic (Mischnick, 2011) and to meet the requirement of relevance of chemistry to everyday life. For the pre-IB school students, introduction to luminescence fingerprinting of food products was also confined to the macroscopic level and demonstration of luminescence effect using lanthanide chelates under UV lamp keeping the task simple and interesting for this age group of students. It has been observed that lack of relevance to real life seems

more for chemistry than physics and biology due to the teaching methods used to introduce chemistry to the students.

9.3 Moving from known to unknown

Research suggests moving from familiar to unknown; start with concrete and move to the abstract, start simple and build up the complexity. The experimental activity was also designed in the similar fashion, as the inhibition time measured for the antioxidant activity in different food samples already measured by a group of students (Farusi, 2009) was presented in the introductory power point to show to the students the relevance of antioxidant activity and inhibition time measured. More inhibition time suggests more antioxidant activity of the food sample. The relevance of more antioxidant activity to healthier food sample was then explained with the results of the experiment, thus extending the concept of antioxidants to better and healthier food.

In addition, students learnt the methods to identify adulteration in food products and to try time-resolved luminescence for the juice samples from different brands.

This approach was also in accordance with Johnstone's (2000) suggested macroscopic approach and it was decided to explain time-resolved luminescence in terms of fluorescence to keep things comparatively easier for students by explaining energy transitions showing photon absorption and emission _ absorption of energy to excite electron and loss of energy in the form of photon, when same electron comes back to the original state. In this time-resolved luminescence, energy comes from lanthanide ion in the chelate (Härmä et al., 2015). Adulterated food products have become an economical and health threat to our society, so this experimental procedure also helps to create relevance of chemistry subject to everyday life.

9.4 Relevance

The goal to carry out this data analysis of the course books was to explore the basis of "everyday chemistry" and its relation to chemistry content of secondary level courses. School chemistry needs to help students understand and use basic chemical concepts but also to relate these concepts to real-world issues and show how chemistry helps in understanding and dealing with science-related issues of everyday life (Childs et al, 2015).

The topic of antioxidants in food was used as context to help students understand chemistry as food is everyday contextual topic and relevant to many chemistry topics in the secondary curricula. This approach is also context-based learning as this research relates subject matter content to real world situations and motivates the students to make connection between knowledge and its applications to their lives (Yam, 2008). This approach in research is aimed to have affective responses of the students to real-life situations and improve the understanding, attitudes and abilities of the students.

The experimental work was based on defining antioxidants, describing examples of antioxidants from daily life and then explaining the mechanism of oxidation and reduction occurring simultaneously in the antioxidants' reactions. Accordingly, the objective of the literature review is to conduct qualitative research of the information mentioned about antioxidants in the textbooks used in the national curriculum as well as international curriculum.

9.5 Inductive content analysis

As no previous studies of the phenomenon in form of book research on the textbooks is available, research method to be chosen had to be inductive content analysis. In addition, the research plan was to deal with the textbooks and content analysis is the method known for analyzing documents.

9.6 Selecting the suitable research method

The first thing was to find the topic of research and then the research method to be used for data analysis. Basic analytical method used for qualitative research on the topic of antioxidants in the Finnish curriculum as well as international curriculum had to be content analysis, as it can be used in all traditional qualitative research methods. (Tuomi and Sarajärvi, 2009, p 91). Content analysis is a systematic research method of analyzing documents and objective means of describing and quantifying phenomena and helps to make valid inferences from data to their context (cited by Elo and Kyngäs, 2008).

Content analysis was to be carried out using inductive approach in the absence of any research previously carried out on the topic of antioxidants included in the curriculum. Inductive approach is always recommended in the absence of sufficient previous knowledge.

9.7 Process of data management

9.7.1 Preparation phase

Before selecting the unit of analysis, it was discussed and decided what information related to antioxidants had to be analyzed in the curriculum books and if there would be no description about the topic in upper secondary school course books, then what other information related to the topic could be analyzed and in what detail.

The unit of analysis had to be selected right in the first step which came out to be antioxidants.

9.7.2 Data collection

For obtaining high-quality data, access to Finnish curriculum books and international curriculum books was made possible with the help of teachers from the university school, Turun normaali koulu and book publishers, Sanomapro had graciously sent the paper version of the books for helping out this analysis and Otava had kindly given access to digital books for a limited time period.

All the books were studied thoroughly to check if the multidisciplinary topic regarding antioxidants has been sufficiently described in the curriculum meeting the requirement of relevance and connecting chemistry with biology and health education.

Inductive approach involves specific to general approach. Firstly, the main topic antioxidants had to be searched and then further categorized by using its examples, mechanisms and examples were further sub-categorized. Data collection was the very first step including all the information related to main heading, categories and then sub-categories. It took long to process the data obtained from different books used in national and international curriculum. Data was formatted into types of files, cross-referred and indexed. Finally, the condensed summaries of the documents had to be developed as part of abstraction step.

9.7.3 Organizing qualitative data

Reading the books thoroughly, it came to knowledge that antioxidants were rarely mentioned as antioxidants in the national curriculum books, rather their examples had been described with their sources and their functions. IB Chemistry book had clearly defined the antioxidants and had clear explanation of their significance and the mechanism of their reactions. The main content was categorized looking for

similarities and differences accordingly to get better understanding. It made the task easier, as to which things to be put in the same category. Simplified expressions were connected and subcategories were formed which led to formation of upper classes and finally the whole concept formation.

All the results of the content analysis carried out had to be then documented as an organized and compressed assembly of all the information presented in IB chemistry and biology books as well as Finnish course books used in the upper secondary school related to chemistry, biology and health education. This part of data analysis is data display. (cited by Miles and Huberman, 1994, p 428)

All the data was carefully analyzed to draw important conclusions. Data collected from book study was read through several times to get immersed in the data and get familiarized with it. (cited by Elo and Kyngäs, 2008). The displayed data was then transformed into conclusions.

9.7.4 Categorization

All the material was openly coded by writing notes and headings in the text while reading it and then material was read through again and again to write down as many headings as possible regarding classes (Hsieh and Shannon, 2005) and headings were finally transformed into categories.

Data was categorized into types of antioxidants mentioned in the course books. IB chemistry had directly mentioned about antioxidants along with the definition, different types of antioxidants and their function as well as mechanism of reaction, hence it was easy to display information but in the biology and health education books, the term “antioxidants” was mentioned in the disguise of vitamins mostly, so the content was categorized forming classes to carry out the word analysis of the content of the document (Tuomi and Sarajärvi, 2009, p 106). It helped to decide which information needs to be in the same category and thus understand it better (see fig 1, p 32).

9.7.5 Abstraction

Antioxidants were mostly described as vitamins, carotenoids or anthocyanins in different course books, with the exception that chemistry IB book directly addressed the topic of antioxidants. Abstraction, i.e. formulating a general description of the

research topic through generating categories was to be carried out. The categories generated were, therefore, vitamins, carotenoids and anthocyanins.

After reading the material again and again and getting familiarized to the content, these classes were underlined, listed and all the information content related to these classes was then subcategorized. Subcategories with similar events and incidents were grouped together as structure, function, examples, sources, applications and reactions and pagination was also carried out as part of analysis to specify the numbers and letters locating specific material in the research content.

9.7.6 Reducing the data

Reducing the information to be analysed by condensing or splitting the data into parts is known as, “reducing the data” (see table 6, p 22)

Original expression	Reduced/simplified expression
An antioxidant is a substance that delays the onset of oxidation or slows down the rate at which oxidation occurs	Definition of antioxidant
Many antioxidants are reducing agents that are readily oxidized bimolecular oxygen or reactive free-radical intermediates, effectively terminating chain reactions and inhibiting other oxidation processes	Antioxidant activity/ Mechanism of antioxidant reaction
Ascorbic acid is a powerful antioxidant and reducing agent capable of donating one or two electrons in biochemical redox reaction.	Example of antioxidant and its reaction
Along with other antioxidants, ascorbic acid is commonly used as additive E300 for preventing oxidative rancidity.	Applications of antioxidants
In living organisms, carotenes and other group A vitamins act as antioxidants, protecting the cells from UV light, peroxides, and free radicals, including a highly reactive “singlet oxygen” produced by photosynthesis.	Antioxidant activity

10 Conclusions

10.1 Description of antioxidants in the course books

The qualitative content analysis of the books being taught in upper secondary schools shows that antioxidants have not been discussed and highlighted well in the syllabus and their importance in the nutrition part of the syllabus is just limited to vitamin C in course books used in the Finnish education system. However, IB chemistry book used in the international upper secondary schools covers most part of the theory related to this topic in detail. The data appears to suggest that IB Chemistry not only defines antioxidants separately but also describes the structure, sources and functions of anthocyanins and carotenoids as antioxidants in addition to vitamins. It also gives the reactions of ascorbic acid showing its antioxidant nature.

IB Biology though does not highlight the chemistry and role of antioxidants but presents at least vitamin C as an antioxidant. Reaction of oxidation and reduction is also explained well describing cellular respiration which is the backbone reaction in reactions of antioxidants.

Though some books used in upper secondary schools in Finnish education portray vitamins as antioxidants like Mooli 1 describing their function and chemical reactions, but still special attention is to be paid to adding more details and knowledge about the role and significance of antioxidants in human health correlating it with the knowledge about free radicals and their harmful effects on our health, so that diet habits could be adapted accordingly to meet the needs of a balanced diet and live a healthy life.

10.2 Relevance of chemistry teaching with the help of antioxidants

Finnish curriculum may add more information about antioxidants as it is the topic of great significance regarding the role of antioxidants in improving human health. Antioxidants are part of our daily diet in form of vegetables, fruits, tea, coffee and many other food products and students need to get the knowledge of chemistry of antioxidants on the macroscopic level and progressively on the microscopic as well as on the symbolic level. This topic, being connection between chemistry and everyday life, would very well attract attention of the students towards chemistry subject.

Students use antioxidants on daily basis being unaware of their true chemistry and beneficial health effects. Getting introduced to antioxidants in the school

education will help students promote the understanding of food chemistry and choose a healthy and balanced diet which will not only show significant effects on their physical health but will also positively affect their progress in studies.

The book analysis reveals that international curriculum keeps focus on the description of different types of antioxidants to some extent starting with the very comprehensive definition of antioxidants in chemistry books. The topic is then extended in the human diet option in biology books as well. Finnish curriculum, however, needs to add the basics about the topic specifically using the terminology of free radicals and antioxidants. Discussing it to the microscopic and symbolic level seems to be over complicated for this age of the students, so it can be simply described on the macroscopic level.

This research work suggests the topic of antioxidants to be suitable for chemistry and biology as well as health education subject taught to the students in the secondary level as well as upper secondary school. Chemistry books explain the chemistry of different processes with chemical equations as oxidation and reduction. Biology says about the types, sources and beneficial effects of all food nutrients. Health education presents more material about energy needs of humans and energy contents of food nutrients. The topic of antioxidants, hence, interrelates three branches of science. It also serves to relate chemistry to everyday life which is one of the main objectives in this research work.

In Finnish curriculum, the definition, sources and types of antioxidants as well as free radicals are missing. When students already get introduced to the concept of oxidation and reduction in this education level, the mechanism of action of free radicals and antioxidants could be easily explained in relation to oxidation and reduction as was found in Bios 3, *solu ja perinnöllisyys*. In Mooli 1 and 2, experimental work related to vitamin C could be presented in relation to antioxidants to make it more life-related issue.

In health education books, starting with food as thought, antioxidants can be explained in a little bit more detail in connection to the balanced and healthy diet where vitamins and their different types have been mentioned in health education books. Students will get motivation to know more about antioxidants if their role in improving the defence system of the body by deactivating free radical reactions is explained. Students will surely be interested to know the precise quantity of antioxidants in every food they eat.

10.3 Reliability of the research and further research ideas

The research topic could be added to the course books of chemistry, biology and health education considering it relevant to everyday life issues while reforming the curriculum for secondary and upper secondary schools. Based on this research work, teachers could choose to introduce the topic relating it to the topic of oxidation and reduction or to food nutrients on the macroscopic level for secondary school students. This topic could be extended further in upper secondary school courses to the microscopic as well as symbolic level.

All the course books have been accessed to get the qualitative overview of the context but it should be kept in mind that the course books are not the only teaching aids used by the teachers nor they tell everything about teaching of chemistry, biology and health education. Therefore, this research does not portray the complete picture nor it covers the whole truth about the antioxidant part in course books. It might need future research requiring more detailed and quantitative information from teachers and students about how antioxidants are treated in the curriculum.

In addition, reliability of this research work might be suspected not to meet the high standards, as only a few books were available for international curriculum investigation and few books for Finnish curriculum investigation from only two publishers. All the course books could not be accessed due to limited resources. The course books have been tried to be searched thoroughly to collect data but there could be chances of missing some information. Therefore, the research work mainly serves to present the ideas how the topic could be used for creating relevance of teaching chemistry with the everyday life.

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Determination of level of antioxidants in food samples and drinks for secondary school students and development of research work for the upper secondary school students using TRL

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Table of Contents

Table of Contents	2
1 Introduction:	4
2 Objectives associated with research work:	4
2.1 Objectives of my research work;	4
2.2 Objectives defined by Finnish National Core Curriculum;	4
2.3 Project specific objectives to be fulfilled in accordance with Finnish Curriculum;	4
2.4 Aims set while planning experimental work;	5
2.4.1 Creating long-lasting connection between chemistry and real life:	5
2.4.2 Adapting healthy eating through chemistry:	5
2.4.3 Briggs-Rauscher reaction;	5
2.4.4 Luminescence fingerprinting of food products;	6
2.4.5 Co-ordination among groups aiming at success;	6
3 Background work:	6
3.1 Choosing the topic of research;	6
3.2 Collection of background information;	7
3.2.1 Free radicals;	7
3.2.2 Sources of free radicals;	7
3.2.3 Antioxidants;	7
3.2.4 Function of antioxidants in the living system;	7
3.2.5 Briggs-Rauscher reaction;	8
4 Initial steps regarding planning of practical work;	8
4.1 Planning Briggs-Rauscher reaction;	8
4.2 Getting equipment and chemicals required for the experiments;	8
4.3 Trying Briggs-Rauscher reaction myself:	9
5 Testing the experimental work with grade 9 students from Turku International school;	9
5.1 Looking for suitable information according to level of understanding;	9
5.2 Amendments suggested for better comprehension;	9
5.3 Communication with the teacher in advance to make necessary preparation;	9
5.4 Testing the experimental work with grade 9 students;	10
6 Planning the developmental work for higher secondary school students;	11
6.1 Planning developmental work after Briggs-Rauscher trials;	11
6.2 X-ray diffraction trials;	11
6.3 Results of x-ray diffraction tests;	11

7	Planning to try luminescence fingerprinting of food products;	11
7.1	Lanthanide luminescence;.....	12
7.2	Trying luminescence fingerprinting myself in material chemistry laboratory;.....	12
7.3	Theory for the developed work for Pre-IB students from Turku International school;.....	13
7.4	Testing the developed experimental work with upper secondary school;	13
8	Feedback from the students;	14
9	Conclusions;	14
10	References;.....	++15
Appendices;.....		16
Appendix 1; Instructions used for grade 9 students of Turku International school, 6 pages.....		16
Appendix 2; Instructions used for pre IB students of Turku International school, 8 pages		21
Appendix 3; chemistry of Briggs-Rauscher reaction.....		28
Appendix 4; X-ray diffraction results.....		29

1 Introduction:

In my research work, I have developed the experimental work for the OpiLUMA laboratory of Chemistry department of the university of Turku. In this research report, I seek to offer an overview of the facts observed during the development of my experimental work.

2 Objectives associated with research work:

2.1 Objectives of my research work;

Objective of my research was to develop work for students which could be used by the teachers and could be tried at Opi-LUMA laboratory and continuation of this work is to develop communication among Opi-LUMA lab and different schools through team teaching. Laboratory work is an established part of courses in chemistry in higher education as chemistry is a practical subject. Providing school students with an opportunity to work in a scientific environment with more laboratory equipment might prove to be an inspiration to attract students' interest towards chemistry subject and take more chemistry courses in university.

2.2 Objectives defined for chemistry teaching by Finnish National Core Curriculum;

While planning my work, I had to consider the general objectives and then specific objectives defined in the national curriculum criteria for chemistry teaching in high school. The Finnish National Core Curriculum provides the frame where the schools construct their own specific curricula. General objectives include improvement in learning environment, the development of cooperation skills and strengthening of concepts.

Main objectives of chemistry include development of scientific thinking, understanding importance of chemistry and its applications in daily life, living environment, society and technology to safeguard environment and human well-being (Opetushallitus, 2014).

In grades 7-9, emphasis is laid on macroscopic level but connection to sub-microscopic level and symbolic models is strengthened to develop abstract thinking of the students. Teaching guides students towards characteristic scientific thinking, use of information, ideas, interaction and assessment of reliability and relevance of knowledge in different situations.

2.3 Project specific objectives to be fulfilled in accordance with Finnish Curriculum;

Chemistry learning specific objectives (Opetushallitus, 2014) to be obtained through my research work are as follows;

T3; To guide the students to understand the importance of chemistry know-how in their own lives, the living environment and society. My work gives information about understanding antioxidants and free radical reactions in living systems and importance of antioxidants in daily food intake.

T6; To guide the students to carry out experimental research in collaboration with others, and to work safely and consistently. Students perform the lab activity independently in groups. Safety instructions are given before starting the experimental work to avoid all the possible hazards.

T7; To guide the students to process, interpret and present the results of its own investigations, as well as to evaluate them and the entire research process. Results are noted down in the table and then graphs drawn to explain the results.

T14; To guide students in their understanding of the basic principles of the properties, structure and changes in the material substance. Basic properties of antioxidants and their defense activity against the harmful activity of free radicals is explained in the beginning through pictures in powerpoint and later in the ending lecture. Luminescence theory is explained through excitation of electron by absorbing energy and then de-excitation losing energy.

2.4 Aims being set while planning experimental work;

2.4.1 Creating long-lasting connection between chemistry and real life:

While investigating the background for fewer students taking chemistry courses in higher education, especially at university level in Sweden, analysis carried out referred especially to role of teacher and lesson planning. Students suggested making chemistry learning relevant to daily life and being more practical and student centered to improve their chemistry experience (Broman & Simon, 2015). I myself was really interested to reveal something interesting about life related chemistry to the students.

2.4.2 Adapting healthy eating through chemistry:

James Beard said, "Food is our common ground, a universal experience." This project is also designed to make students understand the importance of chemistry in the biological systems and develop a sense to realize the importance of healthy, balanced and responsible diet with the help of two different activities.

2.4.3 Briggs-Rauscher reaction;

Students get an opportunity to investigate the relative concentration of antioxidants in different food samples and drinks by using Briggs-Rauscher oscillation reaction. Instructions for the experiments

include information related to antioxidants, foods containing antioxidants, the chemical methods, equipment used and stepwise procedure for the process.

2.4.4 Luminescence fingerprinting of food products;

In addition to the concept of antioxidants, they learn the methods to identify the adulteration in food products as adulterated food products have become an economical and health threat to our society. Students get introduced to the label array technology using unstable luminescent lanthanide labels and monitored luminescence signal resulting in sample-specific fingerprint and perform some part of the tests to become part of the process. Students make calculations from the results obtained and make graphs highlighting the use of this label array technology.

Instructions have different versions for grade 9 students (appendix 1) and a bit modified work for upper secondary school students (appendix 2).

2.4.5 Co-ordination among groups aiming at success;

Research work of this kind fulfils the requirement for chemistry learning environment (Opetushallitus, 2014). Work is planned to be in groups to develop cooperation skills among the students. Concepts strengthen when students get introduced to the topic, get familiarized to the instructions for experimental work and then get the opportunity to analyse the results of the experimental work and finally, reflect upon their learning process through survey forms or questionnaire.

All student groups have their objectives and students try to reach their goals. The task is to observe the difficulties or problems to reach the goals while working in groups. All students participate actively in the group. All the students succeed in their work and learn different experimental techniques.

3 Background work:

3.1 Choosing the topic of research;

While thinking about the topic for my research work, I found that my inclination was more towards food chemistry. I reviewed many topics which are under investigation in Chemistry currently and antioxidants was one of them. Antioxidants have been of great interest for the scientists related to food chemistry, environmental chemistry and many other fields of science in the previous years.

This topic could be used for Chemistry as well as Biology teaching. It removes the boundaries between different subjects as Chemistry and biology, being taught as different fields of science and integrates different branches of chemistry also, as antioxidants and their structures first studied in Organic chemistry and then analytical chemistry through Briggs Rauscher reaction. Finally,

adulteration test is conducted using information about luminescence fingerprinting from material chemistry. It can be used as teaching topic for biology, biochemistry and food chemistry classes as it dictates the reactions taking place in biological systems.

3.2 Collection of background information;

I started looking for some opportunity to make high school and upper secondary school students introduced with this topic and get familiarized with some theory and related lab activity. I had to choose a laboratory demonstration which could be managed easily by the school teachers, in later years, in their own school labs with ease using common and more available reagents.

3.2.1 Free radicals;

An atom or molecule containing one or more unpaired electrons is a free radical. It is capable of free existence.

3.2.2 Sources of free radicals;

Free radicals are generated during normal respiration and cellular functions. “Under normal physiological conditions, approximately 2% of oxygen consumed by the human body during respiration is converted into superoxide anion free radical”. Endogenous sources of free radicals could be mitochondrial respiration, respiratory burst and metal ions reacting with hydrogen peroxide to produce free radicals while external sources include air pollution, tobacco smoking, industrial solvents like chloroform and prolonged exposure to sun, exposure to excessive ultraviolet radiation and treatment with radiation as part of cancer therapy(Dasgupta & Klein, 2014).

3.2.3 Antioxidants;

Body’s antioxidant defence can be classified as antioxidant enzymes, chain-breaking antioxidants and metal binding proteins. Antioxidants interfering with free radicals’ chain reactions are chain-breaking antioxidants which can be water soluble or lipid soluble. e.g. carotenoids, flavonoids and antioxidant vitamins.

3.2.4 Function of antioxidants in the living system;

Dasgupta and Klein (2014) have described oxidative stress to be responsible for many diseases.

“The delicate balance between oxidative stress and antioxidant defence is maintained by the human body for optimal health. Reactive oxygen species and reactive nitrogen species generated during the normal physiological process are also needed in low to moderate concentration for signal transduction and other important biological

functions. If excess reactive oxygen species and reactive nitrogen species cannot be neutralized properly by the body's antioxidant defence, oxidative stress is generated that is linked to many diseases, including cardiovascular diseases, various types of cancer, diabetes, and neurodegenerative diseases”.

Steinmetz and Potter (1991) associated consistent usage of vegetables and fruit with the reduced risk of cancer.

“It is concluded that consumption of higher levels of vegetables and fruit is associated consistently, although not universally, with a reduced risk of cancer at most sites.”

3.2.5 Briggs-Rauscher reaction;

Many other tests have been performed to determine the antioxidants' level in different samples but Briggs-Rauscher reaction attracted me the most due to its series of colour changes showing different phases of the reaction. It could be very interesting for the students in my point of view. Luckily, I found instructions for the lab activity arranged by an Italian chemistry teacher, Gianluca Farusi (Farusi, 2009) for the same age group students. I was quite sure that it could work to give students understanding of the reaction between free radicals and antioxidants.

4 Initial steps regarding planning of practical work;

4.1 Planning Briggs-Rauscher reaction;

I discussed the details of the plan with my research supervisor, Veli-Matti Vesterinen and got approval of my topic. I wanted to try it first myself, so started preparing starting solutions; 4 M Hydrogen peroxide (H_2O_2) aqueous solution, 0.20 M Potassium iodate (KIO_3) and 0.077 M Sulphuric acid (H_2SO_4) aqueous solution, 0.15 M Malonic acid solution and 0.20 M Manganese sulphate (MnSO_4) aqueous solution.

4.2 Getting equipment and chemicals required for the experiments;

All the reagents required were already available in the organic lab and equipment was also borrowed from Opi-LUMA lab. Chemicals required included Hydrogen peroxide, Potassium iodate, concentrated sulphuric acid, malonic acid, manganese sulphate, starch and distilled water. Equipment required included 5 ml and 10 ml pipettes, magnetic stirring plate with magnetic stirrer, 1 litre glass bottles, beakers 100ml and 400 ml, test tubes and burner.

4.3 Trying Briggs-Rauscher reaction myself:

I started the experimental work with fruit juices, jams, distilled water and later tried it with tea, coffee, honey and milk samples. In the final phase of my trials, I also tried vegetable oils and then tried to analyse if reaction could give different experimental results with fresh mango and frozen mango.

5 Testing the experimental work with grade 9 students from Turku International school;

I thought of writing an e-mail to chemistry teacher at Turku international school asking if she could be interested to bring her students to the university for this practical activity. I surprisingly received the positive response to my e-mail in a couple of hours. I needed some information regarding number of students and other to arrange the schedule for the visit.

5.1 Looking for suitable information according to level of understanding;

Using google, Wikipedia and science articles (Farusi, 2009; Qualifications & Support, n.d.), I tried to get some information about antioxidants, free radicals and their interaction in biological systems. I composed all the related theory and chemistry related to the topic for the students and sent it to Vesterinen to read and make corrections according to grade 9 students' level of understanding.

5.2 Amendments suggested for better comprehension;

I had thought of using plastic syringes to take standard solutions to avoid use of glass pipettes by the students but Vesterinen suggested to use micropipettes to make job even easier and safer and get students introduced with use of automatic pipettes frequently used in analytical measurements. He removed the chemical equations showing free radical mechanism in Briggs-Rauscher reaction with the thinking that it would make the theory complicated for this age group. He kindly made other modification in the material and finalized the version of the instructions and feedback form for the visiting group.

5.3 Communication with the teacher in advance to make necessary preparation;

All the supporting material related to theory and experimental work to be done was sent in advance by e-mail to the chemistry teacher for grade 9 to get her guidance as she better knew the level of understanding of her students and could indicate something important to be kept in mind regarding learning process and laboratory work.

Teacher sent a message asking about preparation of the solutions when she went through the plan of experimental work. She wondered if the students get already prepared standard solutions, as students had not prepared any solutions before. I had already prepared the solutions myself

considering safety issues as preparation required handling concentrated solutions and students in grade 9 might not have experience of handling strong chemicals. Secondly, I had to manage time factor as preparation of solutions would have taken longer and students had only limited time duration in OpiLUMA lab, less than 2 hours. Students had only to measure average inhibition time of given food samples with oscillation reaction using readymade solutions.

5.4 Testing the experimental work with grade 9 students;

On 31st November 2016, first group of grade 9 students came to visit OpiLUMA laboratory from Turku International school. Theory about free radicals and antioxidants was presented in the form of powerpoint in the beginning as a pre-laboratory exercise to prepare the minds for learning and to facilitate the concepts. Emphasis was laid on importance of adding more antioxidants in daily food for a healthy life. Briggs- Rauscher reaction was also introduced showing series of colour changes with the help of pictures and video taken during my experimental work.

Safety precautions were clearly mentioned to avoid hazards while dealing with reagents, like use of gloves, goggles and lab coats. Reagents had been kept in the fuming cupboard. For waste disposal, separate plastic bottle was arranged and I told the students to use that carefully for disposing off the reaction products, which needed careful treatment afterwards.

Students had been divided into groups and they worked in co-ordination while following the instructions for the demonstration. Every group had to analyse two samples and each sample was analysed two times to get the average inhibition time for each sample. Using automatic micropipettes was a wise suggestion by Vesterinen as students enjoyed the opportunity to learn new chemistry technique at OpiLUMA laboratory.

I felt that following instructions was a bit difficult in the first exposure to the first sample and they seemed a bit confused in the beginning about steps of procedure. It would have been better to write down the instructions as an outline. Some had been using micropipettes for the very first time. I was all the time there to provide guidance. However, work finished in time when they remembered the steps later and students also got some time to get introduced with centrifuge technique using centrifuge machine for preparing sample solution of semisolid food. Students started taking pictures and recording videos of the colour changes involved in Briggs-Rauscher reaction, which showed that they had really enjoyed the activity.

In the end, teacher and students thanked us for arranging such an activity for school students. They found the topic of antioxidants to be of great interest being related to daily food intake as well as biological systems. Feedback forms filled by the students could help to evaluate later in detail the success of this day.

6 Planning the developmental work for higher secondary school students;

6.1 Planning developmental work after Briggs-Rauscher trials;

I knew from literature review and advice from Vesterinen, that HPLC could be the best possibility for structure elucidation of antioxidants in the food samples and many have already done a lot of work in this regard. I wished to continue with pomegranate juice as it had the most antioxidants among all the fruit juices and read some relevant information of the HPLC tests carried out to evaluate total phenolic content, total antioxidant activity and antioxidant vitamin composition of pomegranate seed and juice(Asmah R, 2014). I could continue the same with less or more modification. But I still wished to do something different from HPLC. I discussed with my supervisor if it was more important to be accurate for structure of antioxidants or it could be something else for upper secondary school students as an introduction to new chemistry techniques at university of Turku. I found some information from google related to chemiluminescence which could be tried. I contacted Mika Lastusaari who had been working with this chemiluminescence, if this technique could work for my samples or otherwise any other technique related to material chemistry.

6.2 X-ray diffraction trials;

The plan was discussed with Lastusaari to perform x-ray diffraction or chemiluminescence if these tests could give any kind of information about antioxidants in food samples. In the supervision of Lastusaari, x-ray diffraction was performed for samples of black pepper, coriander, onion, garlic, coffee(Arabica) and bulgar, first for only ten minutes per sample to see if anything could be observed. Samples were also checked under UV lamp with different wavelengths 254nm, 365 nm and 302 nm but no spectacular observations.

6.3 Results of x-ray diffraction tests;

As analysed by Lastusaari, powdered food samples being amorphous were not well suited for that technique causing diffused background. Bulgar, coriander, onion and black pepper showed some reflections due to crystalline phases and could be more visible if measurement could be made for some hours instead of doing it for few minutes but might explain only the crystalline phases, not the antioxidants.

7 Planning to try luminescence fingerprinting of food products;

Effort to develop experimental work using x-ray diffraction or chemiluminescence did not seem to be working, so the next important step was to start planning some other additional work for the upper secondary school students. Lastusaari thought of trying photoluminescence work done for food

samples done by Harri Härmä and his group at university of Turku, in which samples were measured with photoluminescence. Another measurement had to be taken after some time to see the difference in signals received. With no knowledge of the fact that same test could show something about level of antioxidants, it was decided to get help from Härmä.

Härmä had some valuable suggestions to see my interest for working with antioxidants in spices but extraction of the antioxidants from spices was time consuming and I had to devise an activity before 21 December. An alternate was to try time-resolved luminescence for the juice samples from different brands. Härmä had kindly shared his published research work about lanthanide label array method for identification and adulteration of honey and cacao (Härmä et al., 2015).

After discussion with Vesterinen and approval of the proposal, I went to see the experimental work done under the supervision of Härmä. It took only 30 minutes for analysing one assay in addition to the time required for preparation of the samples. It was ideal time for an additional activity for the visiting group of students in addition to oscillation reaction.

7.1 Lanthanide luminescence;

Photoluminescence is the process of absorption of energy at a specific wavelength and emitting it another wavelength. On absorbing energy molecule gets excited from ground state to a higher level. Molecules return to ground state by producing fluorescence or phosphorescence. (Hagan & Zuchner, 2011) Sometimes excitation energy is transferred to other species e.g. chelated lanthanide ions like Eu^{3+} and Tb^{3+} , from which ion luminescence can occur. It gives significant intense luminescence signals from lanthanide ions which would, otherwise, in the absence of ligand, absorb less energy giving insignificant luminescence signals.

7.2 Trying luminescence fingerprinting myself in material chemistry laboratory;

In supervision of Härmä, three different branded orange juice samples were analysed using equipment provided by material chemistry laboratory including micropipettes, multichannel pipettes, microtiter wells, -96well plates and Labrox TRF plate reader. Assay reagents included terbium containing chelates, Milli-Q water and different branded orange juices. We had to use ten chelates in the start and select the most suitable for the orange juice samples. Two most suitable chelates containing terbium were selected to be used for the demonstration.

As guided by Härmä, it would have been difficult to explain the phenomenon accurately in terms of luminescence to this age group of students and luminescence could be comparatively easier for them to understand by explaining energy transitions showing photon absorption and emission – absorption of energy to excite electron and loss of energy in the form of photon, when the same

electron comes back to the original energy state. In this time-resolved luminescence energy – the photon – comes from lanthanide ion in the chelate.

7.3 Theory for the developed work for Pre-IB students from Turku International school;

All the information related to time-resolved luminescence was written down using information from google images and research paper from Härmä. I sent it to Härmä seeking his kind advice and accurate information about the experimental technique. He kindly offered his time and made valuable corrections to the pdf file as well as added a diagram giving clear picture of the principle of the array technology. He also guided me to show luminescence effect to the students under UV lamp which later proved to be really an amazing part of the demonstration for the students.

This modified version of theory related to luminescence fingerprinting of food products and additional information related to oscillation reaction was sent to Vesterinen with the modified feedback forms who used his teaching skills and precious time to finalize the version for the upper secondary school students.

7.4 Testing the developed experimental work with upper secondary school;

On December 21, 2016, students were given introductory information in the form of power-point about the two different activities and plan of the day. I had written down all the steps in an outline on the board to avoid confusion and the assigned food sample number for each group. It was successful development and positive response to this development was evident from independent working of the students with no frequent questions.

Students had to work in groups. Half of the students started oscillation reaction while other half went to perform time-resolved luminescence activity in material chemistry laboratory to manage space and time in the best possible way and then groups were switched to other activity. I showed them different parts of the chemistry department on the way to material chemistry to let them a bit familiar to the place as well. To wisely manage the time, I had to perform most of the work myself and students kept on watching how to take samples and how they are analysed using labrox plate reader and terbium 2000 delay program. I explained to them the theory involved in energy transitions which is the real cause of changes in signals. They later got the opportunity to use multichannel pipettes individually taking water sample in the wells. I also showed them the luminescence effect by taking chelates containing europium and terbium under UV lamp. Colourful luminescence attracted attention of most of them.

Both activities were managed greatly within the available time. All groups completed their experimental work and filled in the feedback forms. Vesterinen had kindly helped with the supervision of two different groups.

8 Feedback from the students;

Teacher and students greatly appreciated all the experimental work related to antioxidants and adulteration test in food samples. Some of the students wished to know in deeper the chemistry of colour changes which I had not included considering it difficult to be followed by them. Some of the students showed their interest in the topic and wished to try more chelates with fruit juices. Students greatly enjoyed hands-on learning by using multichannel pipettes. Some of them were really excited to try it frequently. Luminescence effect under UV was also amazing observation and they liked the activity.

9 Conclusions;

This experimental work developed under the privileged guidance of my honourable supervisors can be used for the scientific learning in Opi-LUMA laboratory by the visiting students. All the orientation and introduction to the topic, carrying out the experimental work, discussion of the results and reflection for grade 9 can be arranged easily within the time duration for two consecutive lesson hours by the school teachers in their own premises as well, as all the reagents and equipment is easily available in almost all school laboratories. However, for arranging fingerprint luminescence for upper secondary school, an additional half an hour would be sufficient but it needs co-ordination for materials and equipment with material chemistry department.

This kind of work helps students see chemistry as a science at work. It needs accuracy and consistency to make analysis as measuring inhibition time needs careful observation to identify colour changes in Briggs-Rauscher reaction between second and third blue phase. Later analytical part of the work developed for upper secondary school teaches even more accuracy and scientific approach required for advanced analytical processes.

All the experimental and developed work was greatly appreciated by the students and the teacher. I would recommend it especially for this age group students as it clearly defines the relationship between two main branches of science they are studying, biology and chemistry. In addition, it integrates different branches of chemistry like analytical chemistry, organic chemistry, material chemistry and biochemistry. All this material can even be used for students of health sciences like nursing and related subjects relevant to food chemistry.

My research work all could only be accomplished due to valuable co-operation between different sections in chemistry department. My work portrays a beautiful picture of all the efforts and developments being made in different fields at chemistry department of university of Turku. It had been a matter of pride to utilize all the resources and help from within the same department. I believe that even better and more progressive work can be devised for secondary school students and upper

secondary school students helping them understand chemistry concepts clearly and getting familiar with the advancements in chemistry through same kind of co-operative efforts.

10 References;

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Appendices;

Appendix 1; Instructions used for grade 9 students of Turku International school, 6 pages

Determination of level of antioxidants in food samples and drinks

This project is aimed at highlighting the importance of chemistry in the biological systems and develop a sense to realize the importance of balanced and responsible diet. It also allows students to investigate the relative concentrations of antioxidants in a variety of foods and drinks.

Free radicals: Free radicals are atoms or groups of atoms with one or more unpaired electrons. They are electron scavengers and snatch electron from other chemical substances. They are highly reactive and they continue their chain reaction unless stopped otherwise. They are produced during food metabolism or on exposure to smoke or radiation.

In small quantities, free radicals are useful to us, as they play an important role in normal cell processes. But in large quantities they can cause cell damage and impair cell functioning, a process known as "oxidative stress". They may even cause diseases like heart disease and cancer.(2)

Antioxidants: Antioxidants donate electron to free radicals which has now paired electron and can no more damage the cells by stealing their electron. Antioxidants are vitamins, minerals and other chemicals that help protect our cells from the damaging substances, free radicals, produced during normal cell metabolism.

Antioxidants scavenge and neutralize free radicals, thus help to prevent diseases. e.g. vitamin C, E and carotenoids. Other naturally occurring antioxidants include flavonoids, tannins, phenols and lignans. Plant-based foods are the best sources like fruits, vegetables, whole-grain products, nuts, seeds, herbs and spices and even chocolate. Evidence suggests that antioxidant supplements don't work as well as naturally occurring antioxidants in food.

Examples of antioxidants;

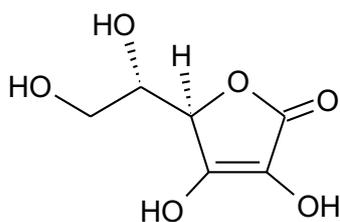


Fig 1; Vitamin C (Ascorbic acid) commonly found in fruits and vegetables

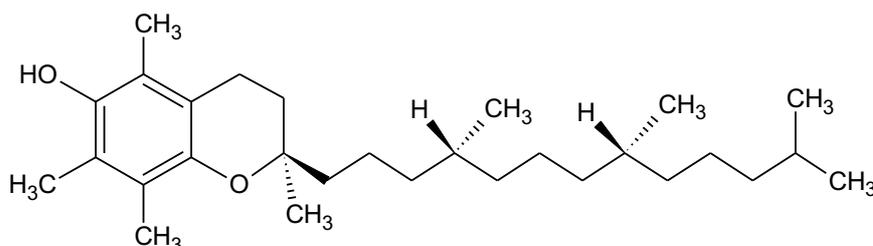


Fig 3; Vitamin E (α- tocopherol) found in vegetable oil, nuts and seeds

Table 1; Antioxidants and food containing them;

	Antioxidant molecules	Food in which antioxidants are found
1	Ascorbic acid	Lemon
2	Lycopene	Tomatoes
3	Chlorogenic acid	coffee
4	Flavanols	chocolate
5	Anthocyanidin with sugar	pomegranate

Briggs-Rauscher reaction: An oscillating chemical reaction showing colour change from colourless to amber to a sudden dark blue is used to determine relative antioxidant concentration in this activity.

Chemicals required:

4 M Hydrogen peroxide (H_2O_2) aqueous solution, 0.20 M Potassium iodate (KIO_3) and 0.077 M Sulphuric acid (H_2SO_4) aqueous solution, 0.15 M Malonic acid ($\text{CH}_2(\text{COOH})_2$) and 0.20 M Manganese sulphate (MnSO_4) aqueous solution, distilled water, food and drink, e.g. samples of wine, teas, infusions; samples of food as aqueous extracts.

Equipment: Magnetic stirring plate with magnetic stirrer, 100 ml and 400 ml beakers, 5 ml and 10 ml pipettes, 1 litre flasks, test tubes, bunsen burner.

Safety precautions:

- 1) Malonic acid and iodine (products of reaction) can irritate skin, eyes and mucous membranes. Fume cupboard is suggested to perform the experiment.
- 2) Sulphuric acid is strong dehydrating agent. Eye glasses, lab coats and hand gloves must be used.
- 3) Disposing off mixture in the end needs reaction products to treat with Sodium thiosulphate, $\text{Na}_2\text{S}_2\text{O}_3$, so that iodine ions are converted to colourless iodide ions.
- 4) Hydrogen peroxide is very strong oxidising agent, so hand gloves, lab coat and eye glasses should be used.

Preparation of food samples (already prepared):

Aqueous solution or suspension is prepared by adding 2 g of food in 400 ml beaker and adding 100 ml distilled water. Stir it with glass rod, decant and pour some amount in a test tube and centrifuge. For drinks like fruit juice, wine or coffee, 2 ml of liquid is added to 100 ml distilled water and stirred well.

Method:

1. Pipette out 10 ml of 4M Hydrogen peroxide solution(A) and transfer it to 100 ml beaker containing magnetic stirrer.
2. Pipette out 10 ml of 0.20 M potassium iodate and 0.077 M sulphuric acid(B) and transfer it to the same 100 ml beaker.

3. Pipette out 10 ml of 0.15 M malonic acid and 0.20 M manganese sulphate(C) and transfer it to the same 100 ml beaker.
4. Place the beaker on top of the magnetic stirrer and turn on the stirrer.
5. Wait for the blue colour to appear for the second time. When the amber solution turns blue for the second time, add 1 ml sample food solution or suspension.
6. Wait for the blue colour to appear again after addition of food sample, and stop the timer. Record the time taken from the start of the second appearance of blue colour to the start of the third appearance.
7. Empty the contents of the beaker into the waste disposal bottle.
8. Repeat all the steps for the other food samples to record the time taken between second and third blue phase.

Addition of food sample after the second blue phase is the stage when non-radical phase is ending while radical phase is about to start. Therefore, longer time interval between second and third blue phase indicates the greater antioxidant level in food sample.

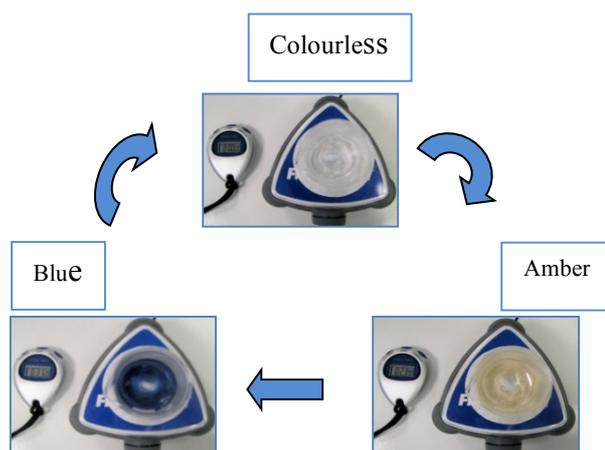


Fig 4; Color changes in the series of oscillations during Briggs-Rauscher reaction

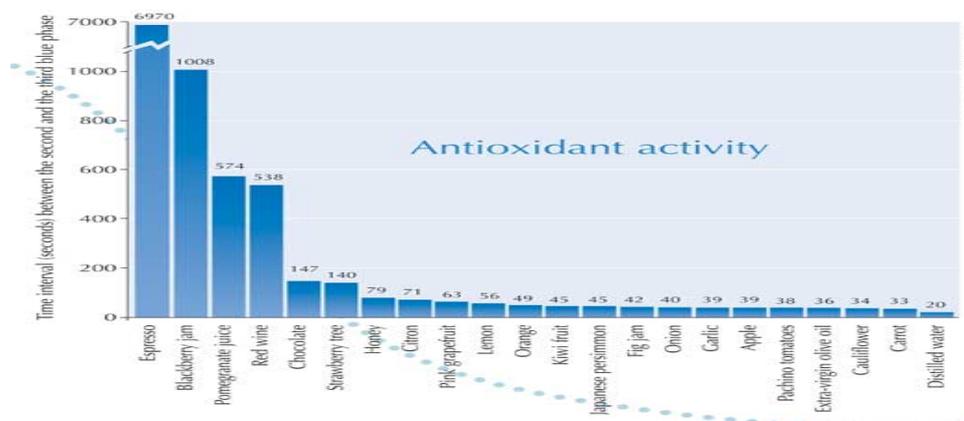


Fig 5; Antioxidant activity in different types of food and drink samples

References; <http://www.scienceinschool.org/2009/issue13/antioxidants>

Observations;

Name of student;

Date;

Table 2; Determination of antioxidants in food samples;

Food samples	Time 1(s)	Time 2(s)	Average time(s)
A			
B			

Write down your observation about the oscillation reaction?

Which sample contains the highest level of antioxidants? How do you know it?

Could you draw a picture to convey what you learned about free radicals and antioxidants?

Feedback about the experimental work: Determination of level of antioxidants in food samples and drinks

Feedback is done anonymously and will be used for the development of work and for thesis work.

Age: _____ Sex; male ___ female _____ don't want to tell ___

Below are the statements about the experimental work you just did. Please read each one and circle a number indicating to what extent you agree or disagree with it.

	Strongly disagree	Disagree	Agree	Strongly agree
Introduction to antioxidants was interesting	1	2	3	4
Experimental work was interesting	1	2	3	4
Instructions were easy to understand	1	2	3	4
I understood the theory of the experimental work	1	2	3	4
I learnt new chemistry technique	1	2	3	4
Visit to chemistry department increased my interest in Chemistry subject	1	2	3	4
I would like to visit Opi-LUMA laboratory again	1	2	3	4
I would like to know more about healthy food and antioxidants	1	2	3	4

Which part of the experimental work was of more interest to you? Which questions arose as you worked?

How would you like to continue this work? What would you like to know more about?

Thanks for the feedback



Determination of level of antioxidants in food samples and drinks

This project is aimed at highlighting the importance of chemistry in the biological systems and develop a sense to realize the importance of balanced and responsible diet. It also allows students to investigate the relative concentrations of antioxidants in a variety of foods and drinks.

Free radicals: Free radicals are atoms or groups of atoms with one or more unpaired electrons. They are electron scavengers and snatch electron from other chemical substances. They are highly reactive and they continue their chain reaction unless stopped otherwise. They are produced during food metabolism or on exposure to smoke or radiation.

In small quantities, free radicals are useful to us, as they play an important role in normal cell processes. But in large quantities they can cause cell damage and impair cell functioning, a process known as "oxidative stress". They may even cause diseases like heart disease and cancer.(2)

Antioxidants: Antioxidants donate electron to free radicals which has now paired electron and can no more damage the cells by stealing their electron. Antioxidants are vitamins, minerals and other chemicals that help protect our cells from the damaging substances, free radicals, produced during normal cell metabolism.

Antioxidants scavenge and neutralize free radicals, thus help to prevent diseases. e.g. vitamin C, E and carotenoids. Other naturally occurring antioxidants include flavonoids, tannins, phenols and lignans. Plant-based foods are the best sources like fruits, vegetables, whole-grain products, nuts, seeds, herbs and spices and even chocolate. Evidence suggests that antioxidant supplements don't work as well as naturally occurring antioxidants in food.

Examples of antioxidants;

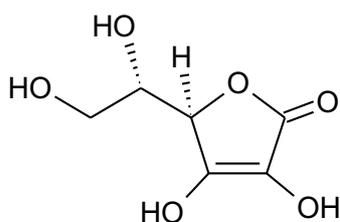


Fig 2; Vitamin C (Ascorbic acid) commonly found in fruits and vegetables

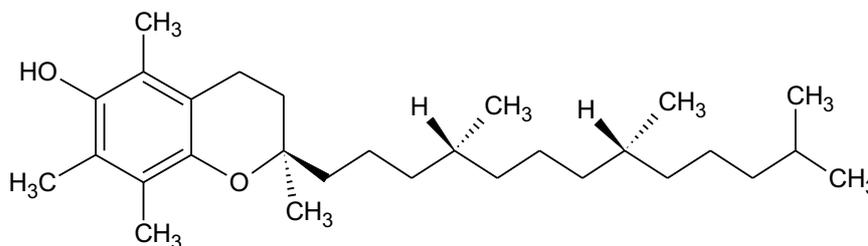


Fig 3; Vitamin E (α - tocopherol) found in vegetable oil, nuts and seeds

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Chemicals required:

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Equipment: Magnetic stirring plate with magnetic stirrer, 100 ml and 400 ml beakers, 5 ml and 10 ml pipettes, 1 litre flasks, test tubes, bunsen burner.

Safety precautions:

- 5) Malonic acid and iodine (products of reaction) can irritate skin, eyes and mucous membranes. Fume cupboard is suggested to perform the experiment.
- 6) Sulphuric acid is strong dehydrating agent. Eye glasses, lab coats and hand gloves must be used.
- 7) Disposing off mixture in the end needs reaction products to treat with Sodium thiosulphate, $\text{Na}_2\text{S}_2\text{O}_3$, so that iodine ions are converted to colourless iodide ions.
- 8) Hydrogen peroxide is very strong oxidising agent, so hand gloves, lab coat and eye glasses should be used.

Preparation of food samples (already prepared):

Aqueous solution or suspension is prepared by adding 2 g of food in 400 ml beaker and adding 100 ml distilled water. Stir it with glass rod, decant and pour some amount in a test tube and centrifuge. For drinks like fruit juice, wine or coffee, 2 ml of liquid is added to 100 ml distilled water and stirred well.

Method:

1. Pipette out 10 ml of 4M Hydrogen peroxide solution(A) and transfer it to 100 ml beaker containing magnetic stirrer.
2. Pipette out 10 ml of 0.20 M potassium iodate and 0.077 M sulphuric acid(B) and transfer it to the same 100 ml beaker.

3. Pipette out 10 ml of 0.15 M malonic acid and 0.20 M manganese sulphate(C) and transfer it to the same 100 ml beaker.
4. Place the beaker on top of the magnetic stirrer and turn on the stirrer.
5. Wait for the blue colour to appear for the second time. When the amber solution turns blue for the second time, add 1 ml sample food solution or suspension.
6. Wait for the blue colour to appear again after addition of food sample, and stop the timer. Record the time taken from the start of the second appearance of blue colour to the start of the third appearance.
7. Empty the contents of the beaker into the waste disposal bottle.
8. Repeat all the steps for the other food samples to record the time taken between second and third blue phase.

Addition of food sample after the second blue phase is the stage when non-radical phase is ending while radical phase is about to start. Therefore, longer time interval between second and third blue phase indicates the greater antioxidant level in food sample.

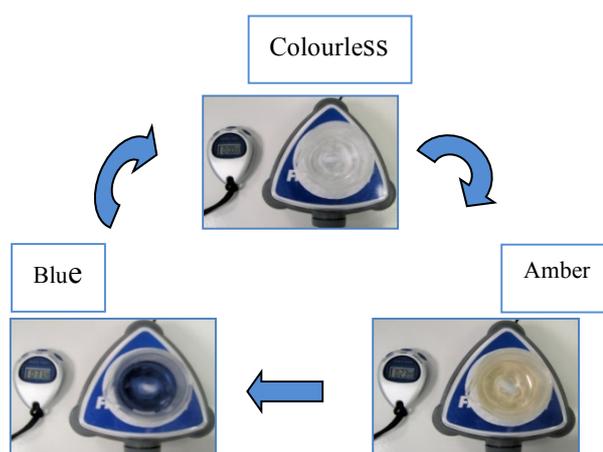


Fig 4; Color changes in the series of oscillations during Briggs-Rauscher reaction

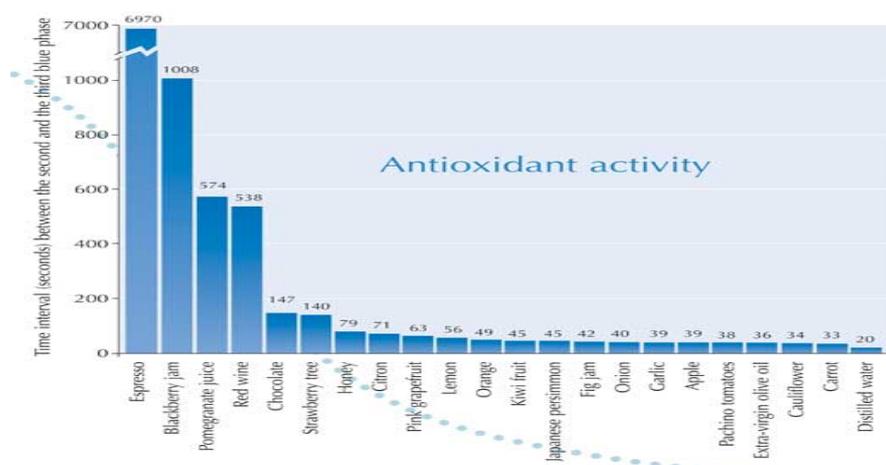


Fig 5; Antioxidant activity in different types of food and drink samples

References; <http://www.scienceinschool.org/2009/issue13/antioxidants>

Luminescence fingerprinting of food products:

This method is used for the identification and adulteration of food products. It is based on a label array using unstable luminescent lanthanide labels and the luminescence signal resulting in sample-specific fingerprint. The method may be used to detect adulterated products, which have become an economical and health threat to our society. For example, olive oil is the most adulterated food product globally and the current testing time for adulteration may take as long as three days. We seek for novel solutions to rapidly screen any potential adulterated food product. The luminescence fingerprinting has the advantage of providing a result economically and rapidly with a user-friendly mix-and-measure type methodology.

Principle: Different lanthanide labels are mixed with a sample and each label is measured for time-resolved luminescence signal. The sample interacts with the lanthanide chelate and gives sample-specific lanthanide complex leading to highly altered luminescence signals.

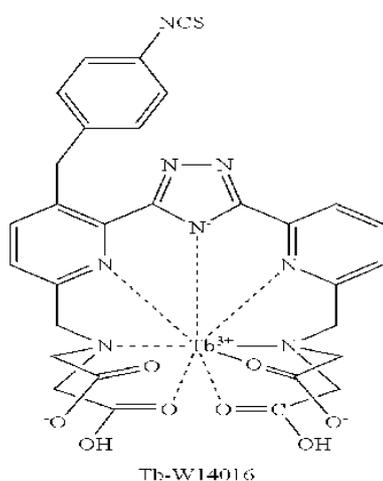


Figure 6; Terbium containing chelate

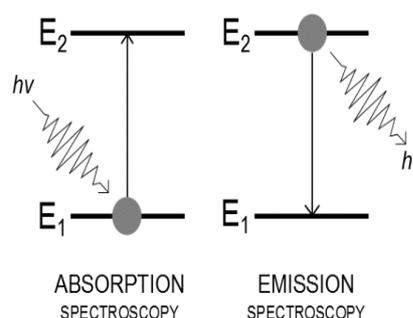


Figure 7; Energy transitions showing absorption and emission

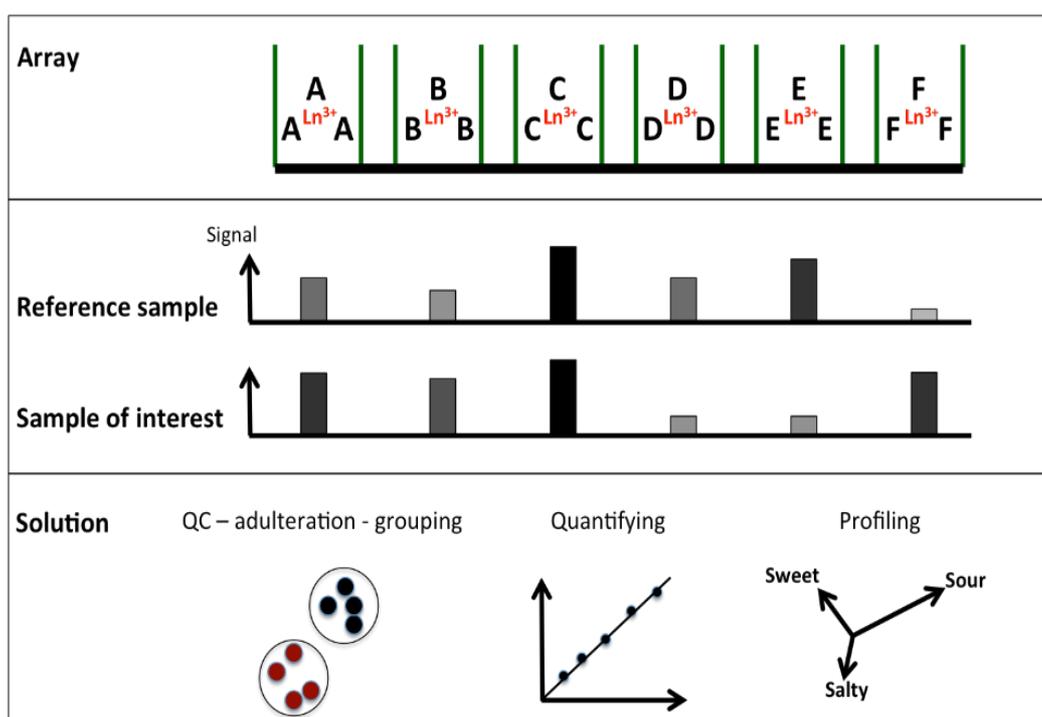


Figure 8; Principle of array technology

Materials and equipment:

Different branded juice samples, terbium containing chelates, Milli-Q water, micropipettes, multichannel pipettes, microtiter wells, -96 well plates, Labrox TRF plate reader.

ID	Name of product	Manufacturer	Texture
1	Orange juice with pulp	Marli Oy	Viscous with pulp
2	Orange juice	Trip	fluid
3	Orange juice	Pirkka Oy	fluid

Dilute the juice samples to 50% using Milli-Q water. Vortex the chelate samples before adding them to sample solutions.

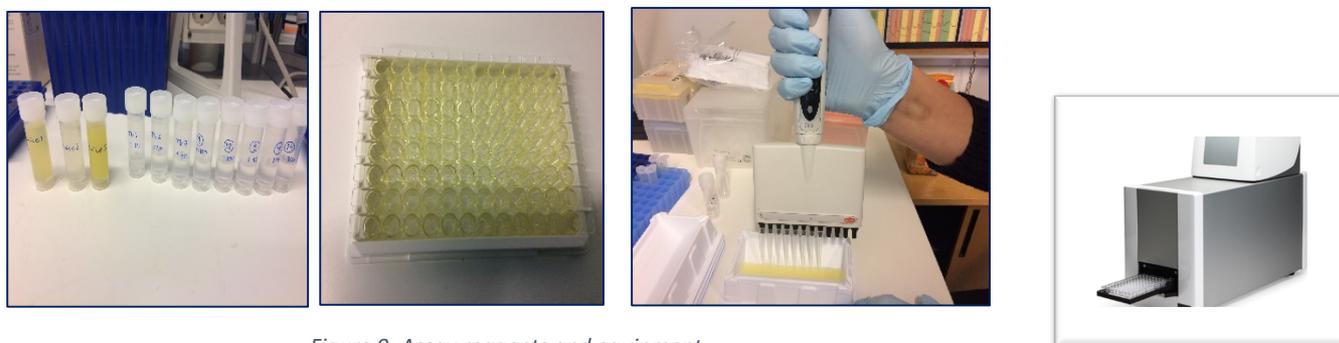


Figure 9; Assay reagents and equipment

In the fingerprinting assay protocol, sample volume of 100 μ l is simply mixed with 10 μ l of the lanthanide-containing solution in the microtiter wells, incubated for 20 minutes and time-resolved fluorescence signals were read by Labrox plate reader using Terbium delay 2000 program.

Students will get the results in excel format about the TRL-signals and will draw a graph comparing the difference in signals for different juices.

References

<http://pubs.acs.org/doi/abs/10.1021/acs.analchem.5b01101>

Observations;(Briggs-Rauscher reaction)

Name of student; ----- Date; -----

Table 2; Determination of antioxidants in food samples;

Food samples	Time 1(s)	Time 2(s)	Average time(s)

Write down your observation about the oscillation reaction?

Which sample contains the highest level of antioxidants? How do you know it?

Observations;(Luminescence activity)

Write down your observation about the luminescence reaction?

How do you know from the results that the juices are different?

Feedback about the experimental work

Feedback is done anonymously and will be used for the development of work and for thesis work.

Age: _____ Sex; male ___ female _____ don't want to tell ___

Below are the statements about the experimental work you have just done. Please read each one and circle a number indicating to which extent you agree or disagree.

	Strongly Disagree	Disagree	Agree	Strongly agree
Introduction to antioxidants was interesting	1	2	3	4
Introduction to determination of adulteration in food was interesting	1	2	3	4
Experimental work was interesting	1	2	3	4
Instructions were easy to understand	1	2	3	4
I understood the theory of the experimental work	1	2	3	4
I learnt new chemistry technique	1	2	3	4
I would like to visit Opi-LUMA laboratory again	1	2	3	4
I would like to know more about healthy food and antioxidants in future	1	2	3	4
Visit to chemistry department increased my interest in Chemistry subject	1	2	3	4

Which part of the experimental work was of more interest to you? What questions arose as you worked?

How would you like to continue this work? What would you like to know more about it?

Thanks for the feedback



Appendix 3;

This scheme was removed from appendix 2 to keep the instructions simple for the students.
(Farusi,2009).

Chemistry involved in Briggs-Rauscher reaction;

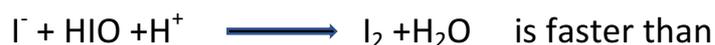
1)As soon as the solutions are mixed, iodate ion is reduced by hydrogen peroxide to produce iodous acid (HIO₂);



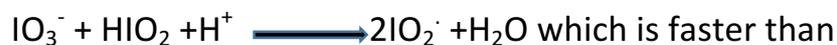
2)The **radical process (amber)** starts, producing hydroperoxide radicals(HOO[·]) and ultimately, hypoiodous acid(HIO);



5) [HIO], [I₂],[I⁻] increase, as



6)On increasing [I⁻], reaction $\text{HIO}_2 + \text{I}^- + \text{H}^+ \longrightarrow 2\text{HIO}$ becomes faster than



7)Radical process stops and starts non-radical process(blue).



Appendix 4; X-ray diffraction results

