Sveriges lantbruksuniversitet
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FOREWORD

I would like to give my warmest thanks to the two key persons of this project: Charlotte Grahn, product development manager at Oatly AB and Lena Dimberg, professor in plant food science at Swedish University of Agriculture Sciences. Charlotte, for first stating this problem in an open-minded-way without any prestige involved and for giving me needed information concerning your products, from the start to the end of the project. Initially, your positive attitude and flexible way of thinking, you gave me the very important first, inspiring input to help me turn this problem into an interesting and complex statement. I would also like to thank Lena for supervision and advice through the whole process – from the first idea to the final report. Thanks for your encouragement and all your guidance in every aspect.

Without your expertise, this Master's thesis would most likely not have come about.

Klara Stenmark, Uppsala, 2013-05-24

ABSTRACT

The aim of this Master's thesis was to find out more about the delimiting quality- and shelf-life parameters over time for liquid oat-based products. According to that, to present applicable subjective and objective shelf-life test approaches at accelerated conditions. The study aimed to gain a deeper knowledge concerning the general and accepted way of performing accelerated storage tests. This study focused especially on products, which suffer from quality failure caused by oxidation, such as liquid oat-based products. The main subject was to present a proper accelerating test method, with potential to be implemented on products with oxidation as the delimiting quality failure parameter to make the production of oat products more effective and economically desirable. Current shelf-life studies cover a long period in time, which prolongs the time and the total costs until new products finally can be launched on the market. An easy stress-test method is desirable to be able to accelerate shelf-life storage measurements in parallel to an actual shelf life-study lasting for the whole shelf-life period. This, in order to get a first indication of whether it is worth to continue with the shelf-life study or not and if the product has the possibility to last and keep a high quality for the whole test period or not. If possibility to implement these kind of accelerated tests, it would be a big advantage.

The easiest, applie able accelerated approach to recommend, is the shelf-life experiments due to Q_{10}-modeling, were two test temperatures in combination with correlation shelf-life estimations will generate a Q_{10}-factor. The intention with Q_{10} -modeling is a way to state the increase in the rate of reaction, when the temperature is increased by 10°C. The value of Q_{10} can be calculated from data of almost every kind of storage test, even if the temperatures are not 10°C from each other. The test temperature should be within a range of 20-60°C. Common test temperatures are10°C apart and experiments should e.g. be conducted at 25, 35, 45 and 55°C. Temperatures of 25°C and 35°C are working as a suitable example, when dealing with autooxidation reactions. At least two test temperatures are required, but three or four is an ideal number for more precise shelf-life predictions. Experimental studies conducted within 60 days period of time seem generate good test results. The Q_{10}-factor based on shelf-life experiments, are used further in order to calculate the activation energy (E_A) for the reaction of interest. The acceleration factor (AF) factor is thereafter calculated by using mathematical equations. The AF factor converts the accelerated shelf-life estimation results back to normal storage conditions. There is most likely time and money to save with a proper accelerated study as it hopefully will give a hint and point out the measureable, limiting parameter and the weakness of a products shelf-life on an early stage. This initial data will give information whether the study should be ended on an early stage to save time and money, or to be continued at full-time length. An accelerated study cannot under any circumstances be the single deciding action to predict a products shelf-life. An accelerated shelf-life study should always be performed in parallel with a full-length study and together with a sensory evaluation program. The sensory evaluation program should be considered as the major limiting parameter, due to the consumers' interpretation, acceptance and preferences.

SAMMANFATTNING

Företaget Oatly har en önskan att korta ner tiden för sina hållbarhetstester för att på så sätt åstadkomma en mer effektiv och ekonomiskt produktion inför lansering av nya produkter. Detta eftersom nuvarande hållbarhetstester tenderar att dra ut på tiden, då de inom företaget i dagsläget utförs vid normal förvaring och då ibland även hela hållbarhetstiden ut. Syftet med projektet var därför att fastställa den gemensamma, begränsande hållbarhetsparametern hos produkter som är baserade på flytande havrebas. Studien har utifrån detta perspektiv fokuserats på produkter som uppvisar kvalitetsbrister pga. den begränsande parametern oxidation. Detta pga. att produkter innehållande en stor andel omättade fettsyror, som tex. havreprodukter, tenderar att reagera med syre vid långtidsförvaring och därmed orsaka bismak. Utifrån den begränsande parametern presenteras s.k. ”stresstest”, där produkten ifråga utsätts för hållbarhetstester vid högre temperaturer under en kort period. Den här typen av tester utförs för att på så sätt påskynda den produktförsämrande och nedbrytande process som annars sker betydligt långsammare i livsmedelsprodukter vid normal förvaring. Möjligheten att eventuellt införa denna typ av ”stresstester” skulle kunna innebära många fördelar eftersom den möjliggör ett snabbare testresultat och en värdefull första indikation på huruvida det är värt att fortsätta med en fullgånget hållbarhetstest hela förvaringstiden ut eller ej.

Studien ämnade därför presentera möjliga ”stresstest” - metoder, dvs. hur den här typen av produkt bör testas sensoriskt och med hjälp av objektiva mätinstrument, tex. vid vilken temperatur etc. för att ge ett bra och tillförlitligt testresultat. Testresultatet används därefter i olika matematiska beräkningar, för att på så sätt beräkna vad hållbarheten är vid normal förvaring. Det finns två olika metoder, Arrhenius ekvation och Q_{10}-modelering. Q_{10}-modeling genererar en Q_{10} faktor utifrån två olika testtemperaturer, vanligtvis 10°C ifrån varandra. Denna är den lättaste metoden att utgå ifrån och därav också den att rekommendera. Syftet med Q_{10} modellering är att på ett enkelt sätt uttrycka ökningen utav reaktionshastigheten när temperaturen höjs med 10°C. Ett Q_{10}-värde kan beräknas ut ifrån resultat från alla typer utav hållbarhetstest, även om testemperaturerna är mer än 10 °C ifrån varandra. Testemperaturen för havrebaserade produkter bör ligga inom intervallet 20-60°C, där hållbarhetstester inom 60 dagar resulterar i bra testresultat. Q_{10}-faktorn används därefter för att beräkna aktiveringsenergin (E_A), dvs. den energi som krävs för att starta reaktionen ifråga. Därefter beräknas en accelerationsfaktor (AF) fram som ett mått på reaktionshastigheten. Denna faktor används för att kunna beräkna och därmed konvertera testresultat ifrån ”stresstester” tillbaka till en beräknad hållbarhet vid normal förvaring.

Det finns troligtvis både tid och pengar att spara med ett väl utfört ”stresstest”, som då syftar till att ge en hint om produktens eventuella svagheter i ett tidigt stadium. Detta ligger då till grund för om hållbarhetstestet skall avslutas eller fortskrida hela studien ut. Ett “stresstest” kan inte under några omständigheter vara det enda avgörande testet. En accelererad studie ska alltid utföras parallellt med en fullängd studie tillsammans med ett sensoriskt testprogram. Den sensoriska bedömningen skall betraktas som den huvudsakliga begränsande parametern utifrån konsumenternas preferenser och därmed produktacceptans.

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1. INTRODUCTION

The patented, today- well-known, oat base produced at Oatly AB is a result of Swedish research technology. The oat-base is the main ingredient in all products appertained to the assortment, produced and offered for sale at Oatly AB. This, patented oat-base is a mixture of oat and water including natural enzymes added, with the ability to break down the starch in the oat. The enzymes ensure the characteristics of the oat-base - the viscosity. The secret is kept in the enzyme mixture including the production process itself. Today, Oatly AB offers a wide range of products made of oat-base to persons who are intolerant to milk and milk products. Oatly AB produces about 13 million liters each year.

Naturally oat contains in general high amounts of unsaturated fatty acids, which explain that many products based on oats have a tendency to easily get oxidized, either enzymatically or chemically. This sensory parameter is the quality parameter to be most aware of, concerning shelf-life and quality failure. The oxidation tendency rate is though depending upon many factors like, the pre-harvest treatment of the raw material and the procedures at the production process. Both external and internal factors of the final product play a significant role, concerning quality and shelf-life estimation of aforesaid products.

It is of interest to hurry up quality and shelf-life procedures and to make the production of oat products more effective and economically desirable. This, since current shelf-life studies cover such a long period in time, which prolong the time and the total costs until new products finally can be launched on the market. An easy stress-test method is desirable to be able to accelerate shelf-life storage measurements in parallel to an actual shelf life-study lasting for the whole shelf-life period. This in order to get a first indication of whether it is worth it to continue with the shelf-life study or not; if the product has the possibility to last and keep the high quality parameters for the whole test period or not. Otherwise it is better to end the study and start with a new one, which will save both time and resources in the end. If there is a possibility to implement these kinds of accelerated tests, it would be a big advantage.

1.1 Aims, purpose and problem formulation of the project

This Master's thesis was performed out of an extensive literature study. The main purpose was to find out more about the production process of oat-base products and its delimiting quality and shelf-life parameters over time. According to that, present applicable subjective and objective shelf-life test approaches at accelerated conditions. In a more detailed context, possible shelf life-testing methods at accelerated conditions, were therefore in focus and reviewed. The study aimed to gain a deeper knowledge concerning the general- and accepted way of performing accelerated storage tests. This by especially focusing on product-tests which suffer from quality failure caused by oxidation, such as in oat-based products. The main subject was therefore to present a proper accelerated test method, with potential to implement on products with oxidation as the delimiting and causing quality failure parameter.

The main question was based on these wishes: Are there any accelerated shelf-life methods available to apply on oat-based products? Are there any studies performed for similar products? How the methods are stated and are they applicable on oat-based products? Which parameter should be considered as the most important quality failure parameter when quality testing oat-based products and also able to measure in an objective way? Which applicable approach is the easiest and how should this stress-test be implemented regarding time and temperature, in a correct manner?

1.2 Method

The literature study was based on books, personal messages and scientific literature. In addition, articles written by authorities abroad or other companies deal- and offering services within shelf-life testing, were used. All literature was found by either searching at the search functions Google, Google scholar, but essentially through the enclosed search engine and database, Primo, available at the SLU library. The search engine through SLU, gave the opportunity to reach many available useful scientific resources such as Science Direct, Scopus, Web of knowledge, Ebrary, Online library, Exlibris, Knovel and Springer. All the information was collected during 2012-2013.

2. BACKGROUND

2.1 The raw product - oat kernels

2.1.1 Quality assurance -storage stability

Since the dawn of time, stored cereals have functioned as a reservoir in times of famine and crop failures. Cereals are relatively easy to store for a long period of time in comparison to dairy products, meat and fresh vegetables. The soundness and quality can however easily be destroyed if storage conditions are not right (Delcour and Hoseney, 2010). Most of the oats produced are used as feed for horses or other domestic animals.

Only quite small amounts of the crop are produced for human consumption around the world. Oats are best cultivated in a cool and moist climate, which explains why most of the oats are cultivate in northern Europe. Cereals in general, are harvested with the caryopsis enclosed, at low moisture content even if it varies due to prescribed circumstances at the time of harvest. The caryopsis is called a "groat" and contains higher fat and protein content than other types of cereals (Delcour and Hoseney, 2010).

The moisture content can vary to a high extent and due to soils and weather circumstances. The water content is of significant importance, which is therefore kept under strict control at each storage unit and is one critical control point at this production level. Oat should have a moisture level of about 11-13%, and should be kept at those conditions to achieve safe storage stability (Sewald *et al*, 2003*).* Time and temperature is also of great importance, in order to prevent microbial growth, especially fungi. Just a few species are prone to attack cereals as surface contaminants. Some species prefer to attack the interior parts though. Fungi species able to grow at these low water activity conditions are mainly *Aspargillus* and to some extent *Penecillium* species. The drying process will ensure evaporation of surface water of the kernels, and at the same time, they are cooled. The kernels will keep on respiring especially at high temperatures, which will accelerate it further. Due to some physical tests, an indication about the kernels physical condition, the deterioration grade and the overall condition such as smells, seen sheen or mate luster can be observed. Stored cereals have to be protected against predators. Mice, rats, insects and bugs are both found within and as surface contaminants of the kernels. Insects can cause big problems, due to direct contamination of the kernels, or even worse, if found by consumers.

According to routine procedures, at first when the oat kernels reach the mill to be processed further, the milling oats are cleaned and foreign materials are removed. Some oat kernels will also be removed due to improperness for milling, such as double oats (the hull covers a second grain). After the cleaning session, the oats are heat-treated or dried. This is done by steam-heating for at approximately one hour at about 93°C in order to lose 3-4% moisture. The heat-treatment will give raise to a roasted flavor and generate hulls easier to remove. In addition, it will also inactivate lipolytic enzymes in the kernel. The kernels are afterwards dehulled and graded according to size. A separator will separate the kernels into large- and stump ones with a high-speed rotor.

2.1.2 Chemical composition

2.1.2.1 Major constituents

The major constituents in oats are proteins and carbohydrate starch (Delcour and Hoseney, 2010). The protein content ranges from 12.3- 22.9% (Peterson and Wood, 1996) and the composition is of higher nutritional value compared to other cereals (Delcour and Hoseney, 2010). This is due to the fact that the limiting amino acids such as lysine, threonine and metheoine are found at higher concentrations in oat than in other cereals (McMullen, 2000). The total carbohydrate content in oat is around 75-80 % of the dry matter (DM), where both starch and non-starch carbohydrates are included. Starch is the main carbohydrate and component of the oat groat (Lászitity, 1998) and the starch content is reported to vary around 45-75% depending on varierity and growth conditions (Asp *et al,* 1992, Peterson and Wood, 1997). The starch is organized in small (3-10 μm) polyhedral granules and consists of approximately 25% amylose and

75 % amylopectin. The gelatinization temperature in oat starch is significantly lower (53-59°C) than other cereals (Delcour and Hoseney, 2010). I will not discuss the major constituents in further detail, since the focus is about the important components affecting oxidative reactions.

2.1.2.2 Minor constituents

The most important minor constituents in oats are lipids, vitamins, minerals, non-starch carbohydrates, phytic acid, enzymes and the phenolic compounds. The non-starch polysaccharides and the lipids, have great impact on the functional properties (Delcour and Hoseney, 2010). The important non-starch carbohydrate group consists of a diversity of polymers. The majority are the β-glucans, arabinoxylan and cellulose (Lászitity, 1998), categorized as dietary fibers. The total dietary fiber content in oat groat varies from 5.0- 13.4% (Asp et al, 1992). The soluble fibers in oats are primarily located in the endosperm cell wall in the sub-aleurone layer. The insoluble ones are found in the bran-tissue outside the aleurone layer (Mälkki, 2001). The main soluble dietary fiber is the polysaccharide, (1→3), (1→4) - β-D-glucan. The reported content of β-glucans varies from around 2-6%, the insoluble ones a few percent higher. The highest amounts of β-glucans in cereals are found in oats and barley. This beneficial compound is associated with the health aspects connected with oat, such as lowering blood cholesterol and a lower risk of diabetes type II (Delcour and Hoseney, 2010). I will not discuss all minor constituents in detail, since the focus is about the important components affecting the oxidative reactions. My main focus is therefore the important constituents taking part in or preventing the lipid degradation reactions.

Lipids

The total lipid content in oat is higher than in other cereals, even if the exact and high amount certainly varies within the crop. Lipids are considered to be one of the minor constituents in oats, but with a great impact of the functional properties (Delcour and Hoseney, 2010). Lipid levels as low as 3% and up to about 12% are known, but 5-9% lipid content is most common in oats. Of the total lipid content 80% is found in the endosperm, instead of localization in the germ and bran as in other cereals (Delcour and Hoseney, 2010). The distribution is presented in table 1 below, where the major fatty acids are the unsaturated fatty acids oleic (18:1) and linoleic (18:2) acids to the percentage of 37 % each. The high amount of unsaturated fatty acids is of great interest, since these are beneficial for human health (Lehtinen *et al*, 2003). Lipids in oat are generally found as storage lipids and are composed of triglycerides and also in the shape of free fatty acids hydrolyzed from them. These lipids are stored as an energy reservoir for germination of seeds. Phospholipids in membranous structures are also found in oat. The fat in the intact, non-germinated grain is though very stable in its natural state, since it is stored in globules protecting it from both free oxygen and enzyme activity. The changes over time at those circumstances are therefore rather small.

Once the oat kernel is milled, lipids will undergo many reactions. If no deactivation of the enzyme system is done, the deterioration process occurs and the lipase activity will get started right away. The fat in the kernel will get exposed to enzymes and fat hydrolysis will occur, generating free fatty acids and mono- and diglycerides. Further deterioration and due to that the chemical auto oxidation of the high lipid content, will generate off-flavors and sensory attributes making the spoilage of an oat-based product a fact (Sewald and DeVries, 2003). Lipid oxidation and development of rancidity are

phenomena often associated with products containing high lipid content, especially unsaturated ones, such as in cereal and cereal products.

Table 1. *Fatty Acid Composition in* Oats

		Fatty acid %		
Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
20	2	37	37	4

(after Delcour and Hoseney, 2010)

Since oat contains such a high amount of linol and linolenic acid, the generation of the degradation product discussed later on, such as the aldehyde 8-, 9-, 10-, 11-hydroperoxides and 9-13-hydroperoxides could be expected as fairly high. The amount of the 9-13-hydroperoxides, can be expected to be up to ten times higher, than the amount of 8-, 9-, 10-, 11-hydroperoxides deriving from oleic acid. Thus linoleic acid tends to react ten times faster with oxygen than oleic acid. The amount of 9-13-hydroperoxides can therefore be expected to be the major contributing compounds to be aware of when detecting the oxidative reaction. This may also explain why the compounds discussed later, n-hexanal (and pentane), are mentioned as the main oxidative indicator in oat product in the literature.

Lipolytic enzymes

Oats differs from other grains due to the fact that oat naturally contains an exceptionally active lipid enzyme system and a huge amount of unsaturated fatty acids, in a disadvantageous combination. The combination of high fat content and an active enzyme activity, might cause some problems concerning stability and sensory aspects when predicting shelf-life on oat-based products. The most troublesome enzyme in deterioration reactions is lipase, which hydrolyzes the ester bindings within the triglycerides (Delcour and Hoseney, 2010). Cereals have lipase activity with different potential, where oats among them has an over representative activity. Another present enzyme, lipoxygenase (LOX) catalyses the oxidation of unsaturated fatty acids. This enzyme is widely spread in cereals (Delcour and Hoseney, 2010; Lehtinen *et al*, 2003), which adds oxygen to the double bonds of polyunsaturated fatty acids (PUFAs), which results in the formation of peroxides. LOX are especially active to linoleic acids and α-linolenic acids (Mellor *et al*, 2010). Another enzyme in oat is the enzyme peroxidase. Peroxidase enables the reduction of peroxides in order to produce mono-, di-, and tri-hydroxy acids and may cause off-flavors as a result. Off-flavors, such as aldehydes are the result of and therefore generated from the chemical autooxidation by a photo-oxidation induced- or by enzymes, the lipoxygenase (LOX) pathway. The activities of these catalyst´s causing the formation of off-flavors are correlated to the availability of water in the oat grain.

Since oat is considered as fairly unstable after process, the shelf life and stability of processed oat products therefore need a proper enzyme inactivation. The especially

high and active lipase activity explains the need for a proper stabilization process. Otherwise the products made from oat can only be stored for a short period of time (Lehtinen *et al*, 2003). Oats are therefore heat treated to enhance the stability of the raw product and the oat-based products made from them. The heat-process will also contribute to the characteristic aroma of oats. Consequently, to inactivate most of the enzymes oat will undergo a steam stabilization process (Dimberg *et al*, 1996). As already mentioned, both lipoxygenase and peroxidase are inactivated due to a steam stabilization process at a temperature above 50°C for several minutes (Sewald and DeVries, 2003). This commercial heat process inactivates most of the enzymes found in oat (Dimberg *et al*, 1996). Steam stabilisation is much more effective, than dry heating in cereal systems. The enzyme peroxidase requires higher temperatures than lipoxygenase to be inactivated, which explains that other enzymes are inactivated if peroxidases´ are (Sewald and DeVries, 2003). A proper deactivation process is one of the specifications to insure on a provider point of view. Cereals such as oat can be controlled for an accurate enzyme inactivation by using an easy colorimetric method (Sewald and DeVries, 2003). Regardless of whether actions are taken to improve the shelf-life in oat products or not, the stability concerning products made from oats, is though shorter than in products made from other cereals having much lower lipid content (Lehtinen *et al*, 2003). This statement can be explained, since the chemical auto oxidation process will nevertheless occur in heat-treated oats by time, even though lipolytic enzymes are inactivated. This reaction will therefore influence the sensory attributes due to generation of off-flavors (Dimberg *et al*, 1996).

Antioxidants

Cereals including oats contain a whole selection of minor constituents, as for example, phytic acid and vitamins. A number of them are reported to have a beneficial, antioxidative effect. Antioxidants prevent oxidation reactions in different systems, regardless of wheter it is in a human body, or in any food system. Free radicals are connected with cell damages in for example the human being, which may cause harmful side effects and diseases. Antioxidants can therefore prevent all kinds of oxidative reactions. A huge range of natural and synthetic antioxidants are found in a diversity of fat-containing food products (Shahidi and Wiley, 2005). Antioxidants are of immense interest within the food industry, because of the need for prevention mechanisms towards rancidity (Sun *et al,* 2011). Antioxidants have the ability to cancel the chain reaction by disrupting the initiation- and propagation phase in the chemical autooxidation. This is done by formation of nonreactive radicals or stable end products that will not continue the reaction further (Niki *et al*, 2005). These antioxidants are hence free-radical acceptors that postpone or totally inhibit the initiation and propagation phase of the autooxidation. The antioxidants react with lipid and/or peroxy radicals and convert them to more stable, non-radical products.

Naturally antioxidants are found in oat. The major antioxidative compounds are vitamin E, phytic acids and phenolic compounds (including avenenthramides). The effect of their activity has been proven both in vivo and under in vitro conditions (Peterson *et al,* 2002). The dominating antioxidant group is however the E-vitamins, mainly tocopherols and tocotrienols. The total amount in oats is about 18.5 mg kg^{-1} DM (Bryngelsson *et al*, 2002). The phenols, avenanthramides, are found at high levels in oat grains (Sewald and DeVries, 2003). The total content is reported to vary from 3 to 300 mg kg^{-1} (Peterson and Dimberg, 2004). Avenanthramides are amides put together with antranilic and

cinnamic acids. So far, 10 different avenanthramides have been found and identified. The most common types are named as 2p, 2c and 2f (Dimberg *et al,* 1996). These three aventhramides also contribute to and are associated with the typical fresh taste of oat. Aventhramides are always found within the oat kernel, but the amount and composition varies according to development stage, fertilization, variety, growing location etc. The largest differences in total aventhramide amount observed are however due to various harvesting years. Differences up to ten times higher content have been observed and reported for same cultivars harvested at a certain place, but at different years (Dimberg *et al,* 1996). This antioxidant system naturally occurring in oat grains could though be destroyed due to thermal process and enzyme deactivation. Some antioxidants can be damaged, which will cause an increase in rancidity and off-flavors in the product in the end. Heat-processing of the raw product will therefore affect both the chemical composition of the primary oat grain and the end quality of the secondary product based on oat. Two types of avenanthramides (N-(4´-hydroxy-3´-methoxy-(E)-cinnamoyl)-5-hydroxy-anthranilic acid and N-(4´-hydroxy-3´-methoxy-(E)-cinnamoyl)-5-hydroxy-4-methoxy-anthranilic acid) found in oats are though considered heat-stable antioxidants. These compounds will thus add some oxidative stability into heat-treated oat grains (Dimberg *et al*, 1996).

3. SHELF-LIFE ESTIMATION

3.1 Basics within shelf-life estimations

The breakdown of a food product is a constant process that often starts before the product has reached the market. Factors such as production process, packaging material, storage temperature, product composition and distribution and transport play a significant role in how long the product can be kept before it becomes distasteful or unsafe to partake (Lawless and Heymann, 2010, Labuza and Fu, 1997, Zoller, 2008). The key concept, shelf-life, mean the requirements for both product quality and safety set together into one concept. Each section and possible analysis method is seen in table 2 below. The center of attention at any quality department that works with shelf-life and stability testing is to maintain a safe, high quality product in accordance to all human senses aimed to meet consumer expectations. Quality changes are expected to arise over time and manifests as physical, chemical or sensory changes. Texture changes, off-flavors taste and perceived color changes such as browning due to e.g. oxidation and/or syneresis, will sooner or later become a quality problem in some way. The safety of a product is always prioritized in front of any other quality parameter. The sensory aspects of a food product are therefore the determinant factor for shelf-life of foods that do not tend to spoil from microbiological changes, such as UHT- treated products (Lawless and Heymann, 2010).

Each quality department at any type of production unit will experience beneficial effects if their sensory control is based on two main basic functions; objective, quality control measurements of the products chemical and/or physical changes, in comparison with a sensory evaluation program. Objective, stability testing is mainly based on chemical testing, such as measurement of the rancidity rate an oxidative indicator, nutrient

degradation, moisture or of physical testing, such as for example the texture. Sensory analysis is often based on subjective testing, even if descriptive analysis performed of a trained panel is classified as an objective test method. Sensory testing in general is based on how each subject is perceived by human senses. Subjects such as aroma, appearance texture and flavor, are sensory tested and evaluated by consumers and by a trained panels. The special types of sensory tests are as follows: discrimination tests, descriptive analysis test and affective testing. These tests, or modifications of them, are often used for sensory analysis. Which test to prefer is depending on what the main questions and objectives are about and on the main purposes of the test (Lawless and Heymann, 2010).

Table 2. *The two main functions within the key concept, "Shelf-life"; both quality- and safety section and their possible analysis indices*

Shelf-life estimations

Quality parameters			**Safety parameters**
Sensory testing	**Chemical testing**	**Physical/instrumental testing**	**Microbiology testing**
Appearance Aroma Flavor Texture and "mouth feel" Color Acoustics (e.g. cracking of biscuits	Moisture Degradation products (Nutrients such as; vitamins**,** minerals, lipids etc.)	Texture/ Viscosity Color Water activity Atmosphere inside the packaging (oxygen, nitrogen, CO2) Pressure inside the packaging	Bacteria (such as E. coli, Lactic acid bacteria, Salmonella, Staphylococcus aureus etc.) Mold/ Yeast

(elaborated from Zoller, 2008)

Heated food products that do not tend to be spoiled from microbiological changes will in most cases be sensory limited due to off-flavors caused by oxidation processes. Oxidation processes are hence the most frequent processes that tend to occur in heated products. The importance of a correct prediction of the shelf-life of a product cannot be stressed enough. This is done by verifying exactly how long the product will keep its sensory attributes before it is oxidized to a level where it is no longer preferable for consumers. The identification of a proper oxidative indicator and its acceptability limits on a consumer basis and methods for shelf-life testing should therefore be in focus. This is done both at actual and under accelerating storage conditions, in order to evaluate and predict storage changes (Decker *et al*, 2010).

As already mentioned, the objective, stability measurements could be performed at both actual and at accelerated storage conditions, sometimes mentioned as direct and indirect methods. The direct shelf-life experiment implicates to run a full-lengths storage study, particularly suitable for fresh products. Actual storage conditions are favorable for fresh products, since quality parameters tend to decompose in a rather short period of time. The storage conditions for that kind of products will be stored and

measured under circumstances simulating ordinary storage conditions. The indirect methods on the other hand, are applicable when to predict a products shelf-life without running a full-length study. The indirect methods can be a big advantage especially for products with long shelf lives, e.g. product suffering from oxidation as a quality failure (New Zealand Food Safety Authority, 2005). The indirect method is a predictive modeling approach by applying an accelerated shelf-life method according to time and temperature. These accelerated tests are based on one main purpose - to significantly shorten the study period by purposely manipulating the storage conditions in order to speed up the deterioration process. This is usually accomplished by manipulating and increasing the storage temperature. The outcomes are then used to estimate the shelf-life under normal storage conditions by conversion due to mathematical models. Mathematical equations are for that reason, used for modeling and predicting a behavior for a certain food product under certain conditions. Theoretically, mathematical predictive models work and are used as a first hint towards a complete evaluation process. The calculations give a first indication of what to expect of a certain product and its shelf-life. Even though these programs provide reliable, valuable and useful data, the information has to be confirmed by an experimentally conducted full-length shelf-life study (New Zealand Food Safety Authority, 2005).

3.2 Basics if setting up a test design

The basic questions when setting up a proper test design, is based on some main questions: 1) What is the main objective of the test? 2) Which test will be applicable? 3) What answers will it generate in the end? Is subjective testing, such as many types of sensory testing, or objective testing such as chemical or physical tests (at actual or accelerated conditions) the best option? These three main types of testing give separate types of answers. Sensory testing will provide more information about appearance, texture and flavor, but in a more subjective way. The objective tests will generate a distinct chemical value. The main purpose of a shelf-life study is to establish specifications of the ideal product and based on that, determine product failure, an acceptance limit or a “cut-off point”, where the test product no longer meets the set up requirements for safety and/or sensory attributes. A product failure is an “all or none phenomenon”, where the product is refused as unacceptable and no longer saleable. (Lawless and Heymann, 2010)

To be able to determine the shelf life and to set up a proper experimental study design, the determining factors need to be identified. Are the causing factors due to the process itself to some ingredient, to a processing step or to packaging? Every significant aspect needs to be taken into consideration - both the physical and the chemical composition of the product, such as water activity, pH, free oxygen, processing activities, raw material, preservatives, packaging and handling/storage conditions (EMSL analytical, inc., 2008). According to Lawless and Heymann (2010) and Zwoller (2008) the basics within shelf life testing include the following procedures:

1) Formulating the purpose of the study
2) Obtaining representative samples
3) Determining the physical and chemical composition of the test product- identifying what may cause the food to spoil or become unsafe
4) Setting up a test design

5) Choosing an appropriate test method and deciding which tests to use (sensory, microbiological, etc.)
6) Planning the shelf life study and choosing storage conditions
7) Establishing the control product or products to which the stored products will be compared
8) Running the shelf life study and conducting the periodic testing
9) Determining the shelf life based on the test result
10) Monitoring the shelf life of the product

When setting up a test design, some questions should be asked. How many samples are needed for each trial and totally for the entire study? At what storage conditions and for how long should the study be carried out? Questions like these work as a foundation when picking the right test design. After formulating objectives, obtaining samples, determining the causing factor(s) (depending on the physical and chemical composition of the test product), setting up a test design and choosing a proper method, the representative samples can be collected and the shelf-life study conducted. The samples will be periodically tested on an equal basis according to the chosen method. The samples should be stored in a way that represents correct storage circumstances for the test products, e.g. mimic normal storage conditions whether it is actual conditions or accelerated conditions. The samples should finally be compared to a control product, e.g. a product with an already known shelf-life, to be sure about the test results, if they are reliable or not. Before the study is conducted, a pre-determined point is usually picked. This is decided to end the study if the product reaches this point early in the process and therefore not seems to maintain safety- and/or quality parameters. The study procedure aims therefore to: 1) Measure loss of quality 2) Reach an endpoint of consumer acceptance 3) Measure the time to reach the endpoint 4) Construct experimental values in a plot. The steeper the slope is at the resulting plot, indicates a fragile food product regarding temperature increase (Gibbons, 1979). The product needs to be tested for a working shelf-life in a daily life, which practically means a product able to withstand poor storage conditions and product abuse in general (EMSL analytical, inc., 2008). Instantly after a new product is out on the market, the process of an innovation and evaluation program takes place. All consumer complaints and reported product defects work as a learning point, worth being aware of and investigating further. The main purpose are to develop an even more beneficial and competitive product. With this in regard, an ideal working quality control function will insure that samples are continuously taken from every batch and shift from the production chain, as well as from the distribution part. Every sample from the distribution chain is taken to follow up and to keep the product quality high according to set standards (EMSL analytical, inc., 2008).

4. SHELF-LIFE PROCESSES

4.1. Deterioration of food products

All foods, raw material or processed food, will get spoiled and deteriorated chemically or by microbes over time. Methods to counteract these losses in quality have been set up ever since these deterioration processes become known to food scientists. Parameters such as tight packages, temperature, time and availability of water and oxygen - all necessary factors for reactions to occur - are important when dealing with shelf-life and food science in general. The main mechanisms concerned in the spoilage of processed foods are 1) Microbiological and pathogen growth 2) Enzymatic and chemical activity causing lipid degradation and color, odor, flavor and texture changes 3) Changes in moisture resulting in changes in texture and flavor.

There are several ways to avoid food spoilage and to control and minimize the favourable conditions for microbial growth. Bacteria, yeast and mold need certain conditions to be able to increase in colonies. Factors like moisture (free water), pH, nutrients, temperature and time for growth, are parameters important for spoilage. These conditions are therefore controlled to prevent microbial contamination and since that, any type of food product contamination. Fresh, chilled products runs the highest food safety risk, since these kinds of products many times suffer from microbiological deterioration and growth of pathogenic organisms and because of that, have the shortest shelf-life (Sewald and DeVries, 2003). Non-enzymatic browning, changes in texture and act of enzymes, are also hinge on these parameters. Additives such as antioxidants and as well the physicochemical characteristics are therefore important parameters to be aware of (Barriuso *et al,* 2013).

4.1.1 Lipid degradation

Dietary lipids have a significant function concerning food nutrition and flavor development in food products. Lipids are occurring either in raw food or added during processing. Oxidation of lipids is defined as the major causing factor relating to quality deterioration that occurs in processed food. The degradation process will, due to a chain reaction, end up in unpleasant primary and secondary oxidation metabolites, such as hydroperoxides and aldehydes, ketones, epoxides, hydroxy compounds, oligo- and polymers (Barriuso *et al*, 2013). These compounds are one of the spoiling parameters to be aware of when trying to predict the sensory shelf-life aspects of foods. This since most of these compounds produce undesirable sensorial compounds (Barriuso *et al*, 2013). Lipids are highly prone to oxidize depending on the rate of unsaturation and due to any possible existing catalytic system available, such as, enzymes, metals, light, heat, metalloproteins or microorganisms. The oxidation process may cause side effects such as off-flavors and loss of essential amino acids, fat-soluble vitamins and other important bioactive compounds (Shahidi and Wiley, 2005).

Deterioration of lipids can occur by two basic reactions: hydrolytic reaction catalyzed by heat and/or enzymes, or chemically - the oxidative reaction mechanism. The oxidative reaction is hence a spontaneous, reaction of unsaturated fatty acid reacting with free

available oxygen (Coultate, 2009). The oxidative reaction is performed and catalyzed out of two possible mechanisms: the photo-oxidation route and the lipoxygenase route. The oxidative reaction demands much lower activation energy to initiate than the heat- and/or enzyme catalyzed hydrolytic reaction, even if both mechanisms are ending up in distasteful compounds. The including lipids and free fatty acids will either react spontaneously and chemically with free oxygen causing oxidative rancidity due to two possible pathways, or by a heat-enzyme induced hydrolyze reaction.

The oxidative rancidity tendency and reactivity grade depends on the structure and the composition of the triglyceride molecule. Each glyceride is constructed by a glycerol skeleton linked together with one, two or three esterified fatty acids attached to it mentioned as mono-, di- or triglyceride. All three fatty acids can show a saturated, monounsaturated or polyunsaturated configuration and be in any position in the glycerol skeleton. Depending on the composition of the fatty acids, lipid molecules are more or less prone to react with other molecules in their surroundings. The saturated fatty acids are quite stable and non-reactive, as no free unpaired electrons are available. The polyunsaturated fatty acids are, on the other hand, exceedingly reactive, due to the fact that these fatty acids might contain up to six double bonds with unpaired electrons. Polyunsaturated fatty acids therefore show a high tendency to easily react with free oxygen, hydrogen or enzymes available.

4.1.1.1 Stability of oil in water emulsions

Concerning emulsions in food systems, factors affecting the oxidation rate in the oil-phase should also be considered. The oxidation rates of unsaturated lipids in homogenous emulsions are dependent on the structure of three phases within the colloidal liquid system. The interior side: the oil droplets, the interfacial material between lipid substances and the aqueous phase. The oxidation rates are said to be higher for negatively charged oil droplets within the system and particle size and droplet concentration may also conduce auto oxidation of unsaturated lipids. The interfacial phase could consist of small emulsifiers and/or of proteins. The third phase, the aqua phase, can include ions, amino acids and macromolecules such as polysaccharides, which may contribute to some stabilizing or destabilizing effect. It has been reported that an increase in the oil-phase concentration, may cause a decrease in the total oxidation rate and as a consequence, also a decrease of volatiles in the headspace of an emulsion (Sun *et al,* 2011).

4.1.2 Hydrolytic rancidity

Hydrolytic rancidity occurs when triglycerides are exposed to water. The presence of water hydrolyzes and split the ester bonds, breaking the triglyceride structure into mono- or diglycerides and free fatty acids. The hydrolytic reaction is catalyzed by either high temperature or due to the enzyme lipase or a combination of both (Sewald and DeVries, 2003). This reaction in general requires much higher activation energy than the chemical reaction and autooxidation mechanism. The water available is present, for instance as frozen water on cold, wet foods and the hydrolytic reaction may occur when deep frying foods are fried in warm frying oil. Unheated fats, however suffer a higher risk of obtaining hydrolytic rancid, since the lipase deactivation has not taken place. In most cases the hydrolytic process goes unnoticed. The enzymatic, lipase catalyzed hydrolytic rancidity mechanism described above, can be found as a result of microbiological spoilage called as microbial hydrolytic rancidity or hydrolysis. This

happens because microorganisms, such as bacteria, can use their lipase to break down fat into smaller units and according to that causes rancidity. The microbiological spoilage (or sometimes wanted fermentation rate) can result in high amounts of free fatty acids (FFA). The amount of FFA is the result of hydrolytic rancidity in food systems. The amount of FFA can be measured, but is not a true measure of the reaction rate, only a measure of the hydrolysis level of a certain product (Sewald and DeVries, 2003)

Most of the FFAs are odorless and tasteless. The long fatty acids esterified and attached to the glycerol skeleton from start, are as FFAs not volatile. This explains why they do not contribute to the odor and off-flavors in the same way. Fatty acids with more than 16 carbons will most likely not affect the flavor of oils since they do not cause a bitter taste before they reach a level of about 2%. In another phrase, FFA will probably not contribute to off-flavors at quite low levels, with the exception of lauric acid which sometimes causes a soapy taste (Sewald and DeVries, 2003). Rancidity and off-flavors, though, can occur if the triglyceride contains volatile, short fatty acids, mainly butyric acid (C4:0) and lauric acid (C12:0). For the most part it occurs in dairy fats. As for example in butter, problem due to hydrolytic release of distasteful butyric and caproic acid derivatives (Coultate, 2009). Butter has ideal conditions for microbial lipase activity in combination with, the fact that it is often left in room temperature. With this in mind, plus water in it and the fact that it is mainly built of triglycerides containing volatile, short fatty acids - the problem is a true fact (Brown, 2011). Butyric acid has a strong odor and can cause an undesirable flavor at low levels (Sewald and DeVries, 2003). The same hydrolytic rancidity mechanism can be a result of microbial spoilage. Microorganism can use their lipase to break down fat into smaller units. Deterioration caused by microbes can be prevented initially during the production process by a heat-sterilization process in order to kill spoiling organisms producing lipase (Brown, 2011).

4.1.3 Oxidative rancidity

The oxidative rancidity reaction is defined as a spontaneous reaction of unsaturated fatty acids in the company of atmospheric oxygen (Coultate, 2009). The reaction is the most common oxidative process and can be accelerated at, for example, higher temperatures (Shahidi and Wiley, 2005). The reaction requires some initial activation energy in order to remove a hydrogen atom from the unsaturated fatty acid. This is enabled by any catalyst such as higher temperatures and the presence of double bonds (Barriuso *et al*, 2013). Another way to describe oxidative rancidity is that: oxygen reacts with a double bound on an unsaturated fatty acid creating primary molecules such as hydroperoxides and radicals (Brown, 2011). Further oxidation steps lead to the breakdown of hydroperoxides and production of carbonyl compounds such as aldehydes. These secondary volatile compounds such as e.g. n-hexanal, have very unpleasant flavors and arise first and foremost from the unsaturated fatty acids oleic C18:1, linoleic C18:2, and linolenic C18:3. The oxidative rancidity mechanism requires quite low activating energy, which hence explains why it occurs easily and frequently on stored products at normal temperatures. The lipid oxidation reaction and the rearrangement of hydroperoxides can be divided into two different main mechanisms: the lipoxygenase route and the photo-oxidation route. In addition to that, the reaction procedure can be divided into three steps, initiation, propagation and the termination phase described in detail below.

4.1.3.1 The initiation step

The initiation step is quite a slow process, which is triggered by any catalyst such as enzyme, light and/or higher temperatures. This is depending on which pathway, the photo-oxidation route or the lipoxygenase route (Brown, 2011, Sewald and DeVries, 2003). The photo-oxidation route occurs when photosensitive compounds with the ability to absorb light, such as heavy metal ions, will be excited to a high-energy condition. Available oxygen in the food system with a triplet ground state reacts with the excided energy of the photosensitive compound and forms a reactive singlet oxygen atom as a product. The singlet oxygen molecules are fairly prone to attack in this scenario to a double bond with unpaired electrons, such as in unsaturated fatty acids. The singlet oxygen molecule reacts about 1500 times faster than a ground state molecule (Sewald and DeVries, 2003). The free-radical initiation state is therefore triggered by a short-lived and fairly reactive high, energy, oxygen molecule ($^1O_2$) unlike the ground state molecule ($^3O_2$). In food systems it mostly reacts with pigments, such as chlorophyll and riboflavin in attendance of light (Coultate, 2009).

The lipoxygenase route is the pathway, when the enzyme lipoxygenase catalyses the addition of oxygen to the double bonds of the unsaturated fatty acids, as seen in the initiation phase below. The fatty acid C18:1 is not a possible substrate for lipoxygenase however. The initiation reaction is hence initially caused by reactive oxygen containing unpaired electrons in combination with a hydrogen atom attached to it, where the very attack of the fatty acid is catalyzed by the enzyme lipoxygenase. The unsaturated fatty acid to be attacked will lose its hydrogen and the out coming products are a water molecules and free radicals, see figure 1 below. The initiation phase gives rise to a small number of free radicals, which are short-lived and extremely reactive and will due to that precede the process; the free radical route. These since the radicals contain highly reactive unpaired electrons seeking a partner in order to fulfill their unpaired electrons (Coultate, 2009).

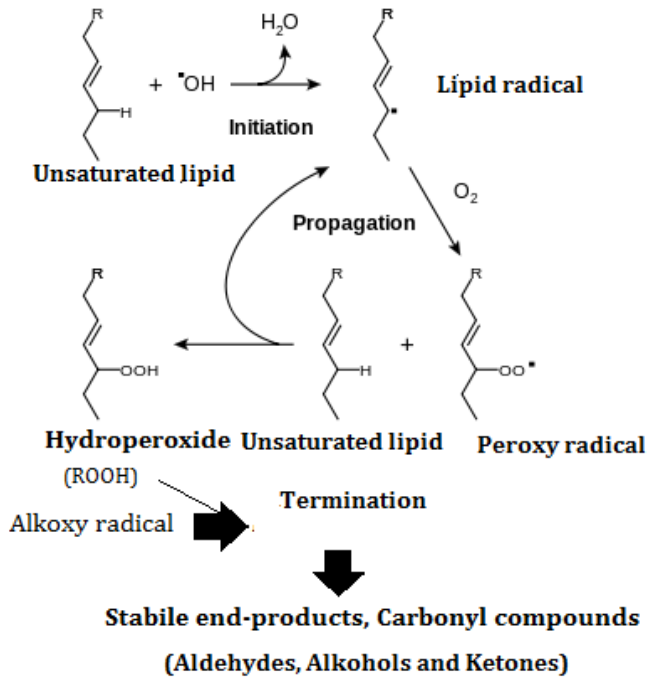

Figure 1. The illustration demonstrates all three phases within the autooxidation (elaborated from Wikipedia, 2013).

4.1.3.2 Propagation step

The initiation step follows the propagation step, an irreversible and self-perpetuating chain reaction phase. The cycle continues, due to the fact that the reaction brings on another new reactive lipid radical, which restarts the process once more. These radicals are therefore both initiators and the final outcome of the propagation reaction in the oxidation reaction (Sewald and DeVries, 2003). The free radical route is therefore the pathway where the autooxidation reaction process generates free radicals as products. The mechanism is clearly defined as a "perpetuating chain reaction mechanism". The propagation step is consequently responsible for the fact that the atmospheric oxygen reacts further with the free radical resulting in a lipid peroxy radical, see figure1 above. The lipid peroxy radicals ar greatly reactive too and reacts further with another unsaturated fatty acid producing a hydroperoxide (ROOH) and regenerating a free radical (Coultate, 2009). The present enzyme peroxidase in for example oats, reduces peroxides further into primary products, hydroperoxides. The reaction causes a configuration shift, were the double bound changes from cis to trans configuration and moves along the chain. Peroxides themselves are tasteless and odorless, but they are further involved in reactions resulting in distasteful aldehydes (Sewald and DeVries, 2003). These compounds are quite bitter compounds responsible for the taste of off-flavors. The hydroperoxides can also be broken down into the free radicals, alkoxy and peroxy radicals, which might contribute to a high concentration of radicals. The high amount of radicals will absorb oxygen in the air to a large extent (Coultate, 2009).

4.1.3.3 The termination step

The termination reaction occurs when the concentration of free radicals is pretty high, and the risk of collisions in between them is unavoidable (Coultate, 2009). The alkoxy radical will give rise to the formation of stable end products such as aldehydes, alcohols and ketones, even if it is still regenerating new free radicals and due to that maintains the chain reaction (Coultate, 2009). The hydroperoxide can in some cases show a cyclic configuration, if it has been reacting with itself. The oxidative rancidity mechanism in food systems is mainly prevented by addition of antioxidants and by elimination of available oxygen in the package.

4.1.4 Hydroperoxides - primary products

The oxidative rancidity process will sooner or later end up in high amounts of peroxides - some rather stable and some not. Highly unstable peroxides are to a great extent formed by compounds that contain conjugated double bonds, which mean single and double bonds in an alternative mixture, such as in unsaturated fatty acids (García-Llatas *et al*, 2007). A part of the peroxides could be kept and are stable concerning their configuration and oxidation rate for a long period of time at room temperature - sometimes for several weeks or even up to a few months. Including metal cations in a food system can for example cause and work as central catalyst and enable configuration shift- and breakdowns of the hydroperoxide molecule to a wide range of secondary degradation compounds (Coultate, 2009).

Oleic acid (C18:1) gives rise to the formation of 8-, 9-, 10-, 11-hydroperoxides in the same quantity. Linoleic acid (C18:2) on the other hand, results in the development of 9-

13-hydroperoxides (García-Llatas *et al,* 2007) and reacts 10 times faster with oxygen than oleic acid. If free oxygen attacks a certain carbon, for example carbon number 13 on the fatty acid molecule, it results in the formation of a 13-hydroperoxides. Hydroperoxides are decomposed further resulting in a broad variety of stable secondary end products, such as different non-volatile and volatile carbonyl compounds and free radicals. These secondary compounds suffer from further oxidization- and degradation reactions and will be of great interest when trying to estimate the oxidative degradation reactions of lipids in food products (Coultate, 2009).

4.1.5 Secondary products

Carbonyl compounds and free radicals are the result of the secondary oxidative degradation process (Shahidi and Wiley, 2005). The carbonyl compounds are e.g. aldehydes, alcohols and ketones. Among them, both volatile and non-volatile compounds can be found, such as hexanal and malondialdehyde (MDA) as main representatives. MDA is one of the most abundant aldehydes generated during secondary lipid oxidation and also one of the most used oxidative indicators. The carbonyl compounds are the major compounds contributing to rancidity and off-flavors in food products. Off-flavors are mainly caused by a variety of specific aldehydes called C6-aldehydes or green leaf volatiles (GLVs). GLVs are generated due to enzymatic activity according to the lipoxygenase (LOX) rout within the autooxidation mechanism (Mellor *et al*, 2010).

Carbonyl compounds cause modest to elevated smells and are responsible for the taste and perception of rancidity in sensorial tests. Identification of these secondary oxidation products is therefore of great significance. This since their development is strongly related to the deterioration of flavor and the perception of rancid taste (Barriuso *et al*, 2013). The oxidative rancidity and off-flavors in foods are often caused by a specific compound within the carbonyl group, depending on the compositional of the product. This specific compound can work as an oxidative indicator to fat degradation in the products (Sewald and DeVries, 2003). Aldehydes in general are produced both via degradation of hydroperoxides and from peroxyl radicals. This through an independent pathway resulting into alkoxy radicals (Barriuso *et al*, 2013). The perception of rancidity of foods is often due to the amount of volatile aldehydes, such as propanal, pentanal, hexanal, heptanal and nonanal (García-Llatas *et al*, 2007, Barriuso, 2013).

4.1.6 Hexanal

The secondary product and C6-aldehyde, hexanal, is created from the13-hydroperoxidisomer derivative (Coultate, 2009) and responsible for rancidity in food products. Hexanal is known to be the most important volatile degradation compound deriving from the chemical, oxidative rancidity mechanism. The amount of n-hexanal can be determined at very low levels in different food products by using a gas chromatographic technique (GC). The method is preferable on products with low fat content, such as cereal and cereal grains and products derived from them. In addition, it is also the most favorable method for shelf-life study designs aimed to detect oxidative rancidity compounds (Coultate, 2009). Hexanal is easy to detect due to low odor threshold and is described as a “grassy” off-flavor in the literature. The hexanal compound is sometimes prone to react further, which may aggravate measures of the specific compound directly. Hexanal has been reported in scientific rapports in different

foodstuff, such as in cooked turkey, freeze-dried chicken myofibrils, fish, and vegetable oils.

4.4 Methods for measuring lipid oxidative products

Numerous of analytical methods are used for measuring lipid oxidation in foods. Many objective tests are based on changes in the initial reactants and since that, detection of oxidation products. Sensory analysis assessment is often favorable, since a descriptive test, for example, provides objective test results due to descriptive scores for specific traits judged by a trained panel. Nevertheless there is no uniform standard method available for detecting every form of rancidity change in all kinds of foods. A diversity of compounds can be used as lipid oxidation indicators in food samples, among hydroperoxides and a selection of aldehydes, are the most frequently used. All of them are markers of primary- and secondary oxidation reactions. The primary and secondary metabolites are given as peroxide value (PV), conjugated dienes (CD), volatile carbonyl compounds, anisidine value (ANV), thiobarbituric acid index (TBA), hydrocarbons or fluorescent products (Decker *et al*, 2010, Sun *et al*, 2011). Both advantages and disadvantages of these methods have to be considered. It is highly recommended to use at least two types of analytical methods when measuring both primary and secondary oxidation products.

The available methods concerning both primary and secondary rancidity products in foods can be divided into four different categories, based on what they really detect in practice: the absorption of oxygen (such as weight-gain methods e.g. preferred for initial phase of the autooxidation/antioxidativ effects), the loss of initial substrates (e.g. changes in fatty acid composition), the formation of free radicals and the formation of primary and secondary oxidation products (Shahidi and Wiley, 2005). Since the focus is all about secondary oxidation products, the techniques for measuring primary oxidation products are not discussed in detail in this text. The rancidity level in food products is mostly given as peroxide value. PV is a great indicator of the initial stages of oxidative changes and a PV of one or less is considered as good (Coultate, 2009). Propanal is often the major marker of oxidation in foods containing high amount of n-3 fatty acids. Hexanal and pentanal on the other hand, are used indicators of oxidation in foods containing n-6 fatty acids. These volatile indicators are often used since they can be easily measured in the samples´ headspace. Hexanal is though more frequently measured as its formation is higher than that of most other secondary oxidation products (Barriuso *et al*, 2013).

Products with a fat content less than 2.5% is preferably tested concerning the total amount of n-hexanal. This since the fat amount is too rigorous in order to run PV or FFA tests, which might lead to misleading, high test results in the end. Samples containing more than 10% fat may lead to proper test results if FFA, PV and hexanal is measured (Sewald and DeVries, 2003). Both PV and FFA are analytical tests which are in general favorably used for oils and fats. Gas chromatography with mass spectrometry detection (GC-MS) is to be preferred to separate, identify and quantify all containing compounds in the sample such as the volatile molecule hexanal. The sample preparation requires long experimental work but the method provides very high specificity and sensitivity and

may detect certain compounds in food samples, at quite low levels. High expenses and complexity in data processing are the main drawbacks (Barriuso, 2013).

5. INDIRECT METHODS

5.1 Accelerated storage tests

Shelf-life studies are needed and important when dealing with food products. These tests provide valuable information about a certain product and its specific lasting period. These kinds of shelf-life tests covering the whole actual test period are time-consuming and costly. Therefore a method to hurry up these tests is desirable. Acceleration-tests and "stress-tests" are developed to give a first hint if the product will last the whole test period or not - a way to hurry the process in every aspect. The result of any type of accelerated-stress test, where a test is performed out of the high temperature storage tests, needs to be compared with results from a real time, long time, study as well. This means in practice that it is not accepted and recognized within shelf-life estimations to just rely on theoretical, mathematical theories, or some accelerated test result by itself. A long time study always needs to be carried out in parallel. This is based on the idea "difference from control", where the long time study works as a control in the end.

The implication of accelerated shelf life test (ASLT) provides prediction of the shelf life at accelerated temperatures able to implement at actual storage conditions by simple mathematics. This is especially of much interest concerning products suffering from oxidation, since it is a rather slow process tending to occur under actual storage conditions. For this reason, it is a great opportunity to accelerate the shelf life by testing the product at conditions apart from actual conditions. The result might allow the extrapolating of data into storage at actual conditions. The ASLT method is dependent of the following principals to be achieved: an exact kinetic descriptive applicable model of oxidation, where the oxidation rate merely varies as a function of the accelerating factor, AF. Other variables are kept constant. Results from accelerated tests are found and calculated from kinetic models where the long time experimental intervals are simulated and premeditated by shorter intervals at higher temperatures (Decker et al, 2010). The basic theory is stated due to the fact that chemical reactions show a predictable behavior, according to time and temperature. The kinetic models are essentially based on the Arrhenius equation, where a known rate constant (k_u) can be initiated from studies performed at different temperatures. If two kinetic reactions will occur with different rate constants (changes at different rate and temperatures); the kinetic constant with the highest value will dominate in front of the other one (Lawless *et al*, 2010).

Even if temperature is the most used and common accelerating factor concerning oxidative reactions, other significant accelerating factors, such as moisture, oxygen concentration, antioxidants and lights, can also be of importance (Decker *et al*, 2011). The huge amount of available data concerning different oxidative stability in food products is though often of less interest. This, since it is difficult to interpret shelf-life data because of big variation in test design, product composition and the definition of the acceptable limits. For example, accelerated studies conducted concerning oxidation in lipids performed under extreme conditions, often have no realistic correlation with

actual storage conditions. The extremes are mainly performed to evaluate the weakness of oxidation concerning different environmental variables. The main purpose of a predictive accelerated study is always to be kept in mind: to evaluate the stability of a product at actual storage conditions (Decker *et al*, 2010). To be able to apply an accelerated kinetic modeling approach regarding time and temperature conditions, the food product has to be able to withstand an increase in temperature. The increase in temperature at the accelerated storage test, aims to as big an extent as possible, at only leading to an increase in oxidative reaction. Some complications can though arise, especially in multi-component products at high storage temperatures. This according to the fact that lipids, carbohydrates and proteins could react with each other resulting in new unwanted compounds (e.g. Maillard reaction) and those may stand in as pro- or antioxidants causing a false result. Additional possible changes, seen at thermal treatment responsible for product spoilage, can for instance be pigment bleaching caused by oxidation and physical changes like phase and viscosity transformation.

5.1.2 Sensory testing

Sensory specialists may be familiar with the phenomenon that the changes shown with accelerated shelf-life tests according to time and temperature changes is not always the same as changes shown in products stored at normal conditions (Robertson, 2006). For instance, drawing a conclusion regarding shelf-life from heating a frozen sample, makes no sense. Some kinds of food will follow neither of these predicted models due to the fact that other processes may occur too, like phase changes. The sensory result from any type of accelerated-stress test has to be compared with the outcome of a full-time study. The sensory aspects and the measurements of objective shelf-life parameters need to be tested towards each other as well. According to a study performed by Lee *et al* (2002) this theory was used in practice. Samples were evaluated sensorially by using descriptive analysis in combination with instrumental analysis (GC analysis of hexanal). The major objective was to find out when these methods correlated in sensory attributes and when instrumental measurements of hexanal correlated. A study conducted by Grosso *et al* (2002) pointed out the importance of predicting consumer acceptance due to consumer ratings out of a descriptive analysis in addition to a consumer evaluation program. This was proceeded in order to figure out when the data correlated and how long the shelf-life should be estimated to be.

The sensory descriptive test is often the sensory test to prefer, since it is the most complex and sensitive of the sensory evaluation tools existing (Kilcast and Subramaniam, 2000). A descriptive test provides a detailed description of the sensory attributes including the intensity perceived for each attribute in a product. It is performed by a trained sensory panel, who can distinguish when the product no longer meets quality requirements. It should be done due to exact circumstances, and that is why ISO-standard (ISO8587) is favorable. This is used to make sure that the results are reliable and that the test is conducted in a correct manner. Regularly, an expert panel, internal or external, is used to carry out a descriptive sensory analysis. This is performed in order to describe how sensory attributes might be responsible for consumer complaints and rejections. Affective sensory testing by using a consumer test and hedonic scale can also be of much interest. The values obtained on a hedonic scale can be used to determine the endpoint of consumer acceptance on a specific product. If a 9 point, hedonic scale is used, a food sample with a value of 5 means in practice, neither that it is liked nor disliked. If a value

of 4 is obtained, it should be considered as faintly disliked. Therefore, values lower than 5 on a 9-point hedonic scale, can be considered as a levels indicating that the food is unacceptable for the consumer. Irrespective of chosen method, the main question to be answered is hence when the theoretical and/or accelerated test results will synchronize together ("cutoff point")? The sensory aspects are judged by a panel, which is trained to recognize when the sensory aspects will coincide with the shelf-life test results. The sensory panel will determine when the product no longer meets the product requirements for sensory aspects. The sensory information about already tested products will be used as valuable information when predicting and testing shelf-life regarding new products with quite similar constitution (personal message, Karin Wendin, SIK).

5.1.3 Definition of acceptance limit

Products suffering from quality deterioration during storage, such as oxidation, will sooner or later become unpleasant due to a high rancidity grade. A certain acceptability limit in such products needs to be exactly specified with the main purpose to limit the number of released rancid products on the market and due to package claims, such as "best before" date. Concerning acceptability limits in general, it is much easier if the acceptance limit is compulsory, specified and regulated by authorities on a national or international basis. If that was the case, the producer would simply need to follow current legislation and guarantee that the acceptability limit is kept and not exceeded under any circumstances. According to current regulation, the producer must guarantee product conformity to any claims written on the package. This is required even if the claim is on an optional basis from the producer and not compulsory due to the basic legislation.

In fact, the acceptability limits are not regulated by any national or international authority, concerning rancidity. The adopted acceptability limits are completely free and up to the producer to set its standards concerning quality assurance. The acceptability limit is mainly decided and managed from former experience at the company, when trying to predict a certain product´s performance. Over- and underestimation of the limit is of course a risk when predicting a behavior for new products. On the other hand, previous experience concerning already known products makes the process much simpler. Sensory testing is often the easiest approach and a favorable indicator of product spoilage due to oxidation. The producer has to make a decision, when the product reaches the acceptable limit, a "cut-off point" and is significantly distinguished from the fresh product on a sensory basis.

According to a quite ancient study, the inception of rancid odors in oat cereals, was found to initiate when the hexanal concentration increased to a level of between 5 and 10 ppm (parts per million). In addition concerning the sensory evaluations attributes, the study proved a correlation with a hexanal concentration between 0.3 and 5 ppm (Fritsch and Gale, 1977). Another example when predicting consumer acceptance ratings directly associated to the degree of product rancidity is a study concerning cracker-coated and roasted peanuts performed by Grosso *et al* (2002). The cut-off point for acceptability of stored nuts was observed at a hexanal content of 5.39 μg/g in cracker-coated peanuts and 7.40 μg/g in roasted peanuts. The obtained rancidity and

flavor intensity was seen at values higher than 27.4 μg/g in cracker coated peanuts and 36.2 μg/g in roasted peanuts.

The oxidation attributes gathered from the sensory panel, and many times also responsible for consumer complaints and rejections, is converted to either acceptable or non acceptable binary data. Affective sensory testing by using a consumer test and hedonic scale can be of great significance. A value adopted from an affective, consumer acceptance test lower than 5 on a 9-point hedonic scale, can be considered as a levels indicating food unacceptable for the consumer. This since the results achieved by a trained test panel are in many cases not the same, as experienced by the consumers. Sometimes, the cut-off value chosen by the company is not correlating to what is perceived by the consumer. The consumer rejection and risk monitoring is mentioned in percentage and is used as an indicator of acceptable limit. An average risk level, 50% consumer rejection, is often chosen as a reasonable percentage. The definition of acceptance limit is a quite costly and time consuming and there are no known possibilities to speed up the process (Decker *et al*. 2010)

5.2 Setting up a test design for a product which suffer from oxidation

To achieve a cost-effective test design suitable for products undergoing oxidative reactions, a systematic and schematic way of doing the shelf-life determination is of great importance. As seen in table 3 below, simple, possible strategies able to imply, are presented when evaluating shelf-life of foods suffering from oxidative and rancidity mechanisms. To begin with it is important to set the definitions of the acceptable limit of the causing factor. The next step is to determine the correct oxidative indicator. When the oxidative indicator is set, it is possible to predict the shelf-life according to mathematical kinetic modeling calculations. As already mentioned shelf-life evaluation could be performed at both actual and accelerated conditions. Since oxidation in food products suffering oxidation is a rather slow process, an accelerated approach is the best option. The accelerating condition is therefore constructed and conducted in order to speed up the deterioration process. When the acceleration factor is computed, the oxidation kinetics modeling needs to be evaluated under accelerated conditions. This is done in order to get to know the effect of the accelerating factor (AF) regarding the oxidation rate. Obtained information from kinetic models is then converted to valuable data able to extrapolate at actual storage conditions (Decker *et al*, 2010).

Table 3. *Basic procedure to follow when shelf-life testing products at actual an/or accelerated conditions*

1. Definition of acceptance limit/ "cut-off" point
2. Identification of oxidative indicators (sensory and/or chemical)
3. Shelf-life testing

3.1 Shelf-life estimation under actual storage conditions	3.2 Shelf-life prediction under accelerated storage conditions
• Oxidation kinetic modeling under normal storage conditions; full lengths and normal storage temperatures • Shelf-life estimation	• Oxidation kinetic modeling under accelerated conditions; accelerated time and temperatures • Modeling the effect of the accelerating factor on oxidation rate

(elaborated from Decker et al., 2010)

5.2.1 Experimental design

The concentration and quantity of n-hexanal in low fat foods, such as in emulsions, can be detected within 10 minutes by the GC analysis due to determination of the total quantity of volatile compounds. A homogenous portion of the sample must be collected (Sewald and DeVries, 2003, Fritsch and Gale, 1977). The sample is preferably kept cold to minimize the risk for oxidation before analysis. The test temperature should be set within range of 20-60 degrees and at least six samples should be analyzed. The more polyunsaturated fatty acids present within a food sample, the lower the test temperatures should be, when performing a test to confirm the oxidative stability (Frankel *et al*, 2005). In practice, therefore oils of vegetable origin should be tested at temperatures lower than 60 °C. According to Gomes-Alonso *et al* (2004) the formation of primary and secondary oxidation products were observed in the temperature range between 25 and 75 °C. Common test temperatures are often 10 °C apart and conducted at e.g. 20, 30, 40, and 55 °C. A control sample, stored at 0 °C, is preferable. Higher test temperatures require more frequent testing in general. Analysis each week is commonly performed. Some accelerated study samples are according to the literature, collected every third day (El-Magoli *et al*, 1980). Each testing temperature should have at least six data points over time to make the study results statistically reliable. At least two

temperatures are required, but three or four is an ideal number for more precise shelf-life predictions (Sewald and DeVries, 2003, Hough *et al*, 2006*)*.

An accelerated shelf-life study (30 days at 25, 35, 45 and 55°C) performed by Su-Chuen *et al* (2007) proved that quinoa was oxidative stable for the 30 days according to the amount of free fatty acids, hydroperoxide and hexanal conte. Storage time and temperature did have significant effect concerning all parameters. The largest change in hexanal was seen in samples stored at 25 and 35°C. The increase was seen from day 6 to day 18, followed by a gradual decrease. A study conducted at a temperature over 50°C is of no interest, since the formations of hexanoic acid out of hexanal tend to occur at these higher temperatures (El-Magoli *et al*, 1980). In addition, also at higher pH, n-hexanal can be converted to n-hexanol by alcohol dehydrogenase (ADH). This conversion of hexanal has though small impacts on sensory attributes in food products (Mellor *et al*, 2010). Quite recent studies concerning shelf-life at accelerated conditions in peanuts, showed a very small increase in hexanal content within the first 30 days. This may point out the importance of a longer study, in order to draw conclusions of the increase in the total hexanal content (Grosso and Resurreccion, 2002, Lee and Krochta, 2002 and Lee *et al*, 2002). A shelf-life study concerning oxidative stability could be carried out for 45 days and even up to above 100 days according to previous studies. A study seems proper and generates good test results, if the study is conducted within 60 days (Grosso *et al*, 2002, Lee *et al*, 2002). An increase in hexanal content has also been found during a long period of time, for over 30 weeks, which may defend longer studies as well (Lee *et al*, 2002)

5.2.2 Sample preparation and analysis method

A possible sample preparation method could be as follows: A homogenous portion of the sample is suspended and mixed with boiling water containing an internal standard e.g. 4-heptanone (Fritsch and Gale, 1977). The sample is then heated for a specified amount of time. Then a sample of the headspace (the volume left at the top of a filled container) is taken and injected into the GC analyzer. In the same pace as the oxidation increase, the amounts of volatiles increase (Sun *et al,* 2011). The hexanal released is detected and quantified via comparison of the internal standard (Sewald and DeVries, 2003*)*. A high amount of aldehydes with a small molecule weight indicates an extended oxidation rate in general. The method is of high sensitivity and can reflect the oxidation deterioration of lipids in food products (Sun *et al*, 2011).

5.4 Identification of oxidative indicator

The surveyed objective data of an oxidative indicator is aimed to be set in relation to a proper subjective sensory analysis demonstrating consumer perception. The main purpose of these objective measurements is therefore the identification of one proper, single oxidative guide value. The central function is to correlate both consumer perception and rejection on a sensory basis towards an objective, measurable chemical indicator. The chemical indicator has to be measureable and easily correlated to sensory observations. A possible procedure to follow when identifying an oxidation indicator is seen in Figure 2 below. The most important compounds undergoing oxidative reactions potentially correlating with consumer rejection, are unsaturated fatty acids and consequently a number of sensory attributes correlating to them. To be able to evaluate the lipid oxidation, the method is based of measurement of either primary or secondary oxidation products of unsaturated fatty acids. Other possible measurements are for example the detection of sensory compounds responsible for off-flavor formation.

Beside unsaturated fatty acids in fat and oils, other selected compounds like pigments, such as carotene and lycopene indicators can be responsible for consumer rejections due to unlikeable sensory attributes as color fading and off-colour in the product. These indicators usually detected sensory or by a spectrophotometer are said to be the quickest and easiest method used for consumer perception and rejection. Once the identification of the best correlating oxidative indicator is decided, further procedure is based on the following questions:

- How does the amount of the oxidative indicator and consumer acceptability change during food storage?
- What value of the oxidative index corresponds to the sensory acceptable limit?

Answering these questions is a key function in generating an easy method for shelf-life estimation minimizing the practice for costly consumer testing. The aim is to determine an index value of oxidative reactions, a "cutoff value" of the indicator which correlates with consumer rejection. It needs to be pointed out, that each oxidative index and the acceptability by consumers only correspond at a certain storage temperature. The consequence is that all tests, both measurement and sensory testing, need to be performed for each storage temperature in order to get reliable data (Decker *et al*, 2010)

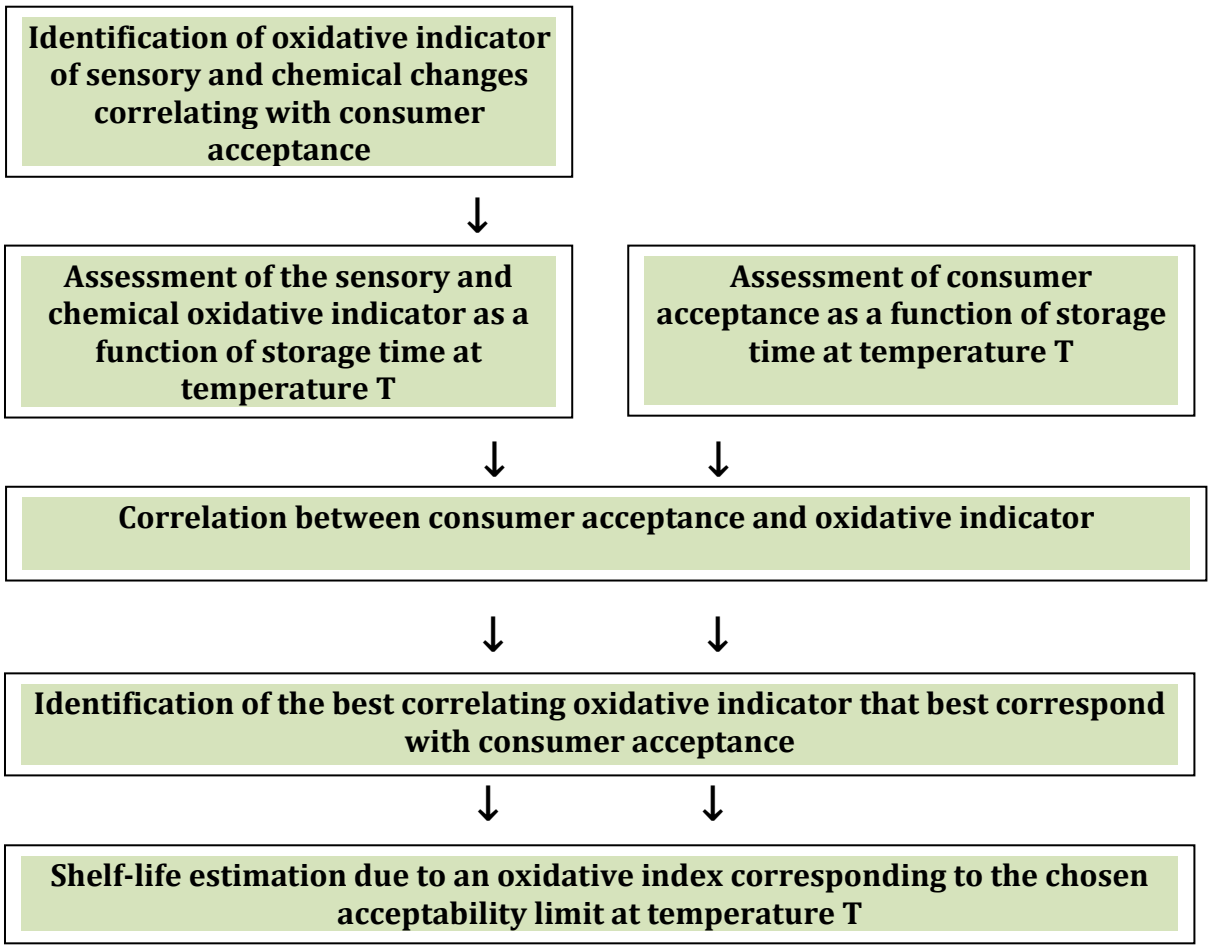

Figure 2: Procedure for identification of an oxidative index corresponding to consumer acceptance (elaborated from Decker *et al*, 2010)

5.5 Kinetic mathematical models within shelf-life testing

Shelf-life prediction is based on reaction kinetics where the acceleration of the deterioration rate is direct time- and temperature dependent. The deterioration processes can depend upon physical, chemical, microbial or biochemical processes (Decker *et al*, 2010). The composition of the product and the environmental conditions such as available oxygen in the atmosphere, humidity and ambient light etc. are important factors that need to be taken into account when trying to predict a kinetic relationship. An accelerated shelf-life test is possible to implement on any type of deterioration process that can be measured and can be expressed quantitatively by a proper model. The chosen model aims to state the changes in a shelf life test by expressing the value of a certain marker of interest. In order to apply a kinetic modeling regarding deterioration processes, a valid model used for storage test should include all factors that may affect the reaction rate - both positional and environmental factors. The kinetic modeling method is the most common method for accelerated shelf life testing (Kilcast and Subramaniam, 2000). Some kinetic mathematical models are used to express kinetic relationships at both actual and accelerated conditions. Calculations enable conversion of accelerated data into useful data at normal storage conditions. Basics within kinetic modeling are the behavior between the changing, measureable, quality assurance, parameter (Q) and time (t), which is known to be linear as seen below in eq.1(Sewald and DeVries, 2003). The $\delta Q/\delta t$ is the change in the measurable, quality indicator mentioned as Q. The rate constant, *k* is mentioned in an appropriate unit and *n* is the order of the chemical reaction due to packaging and temperature etc. Quality loss (Q) in general therefore follows the relationship (Sewald and DeVries, 2003, Labuza and Fu, 1997):

$$\delta Q/\delta t = k(Q)^n \quad \text{(eq. 1)}$$

In food systems, the chemical reaction is set to either zero, first or second reaction order depending of the type of chemical reactions that are expected to occur. The first order reaction is the predominant reaction order in food systems (Toledo, 2007). Rancidity reactions are examples of first order reaction since it is a degradation reaction. The rate of quality decrease is exponential. Plotting the log scale values versus time, makes the experimental data more easily interpretable. Both zero and first order reactions allow determination of the rate constant (k) from the linear relationship (Lawless and Heymann, 2010). The rate constants show either a positive or negative value depending on if the quality indicator increases or decreases in relation to time (Decker *et al*, 2010). These statements are explained further in the text below. Once the k value, the slope, is determined, the activation energy (E_A) can be determined by using Arrhenius equation. The zero order reaction curves result on the other hand, in a linear plot and are for example seen when performing shelf-life studies at actual storage conditions. At these conditions, the kinetic model should only contain factors that change during storage (Kilcast and Subramaniam, 2011). The rate of loss is known as constant in shelf-life (SL) at actual conditions. Second order reaction is on the other hand dependent on the level of two factors (Decker *et al*, 2010).

5.5.1 Arrhenius equation

Among many potential accelerating factors, temperature is the most commonly used within ASLT (Ragnarsson and Labuza, 1977). Arrhenius equation is widely used (see eq. 2 and 3 in appendix). A chemical deterioration process requires a certain amount of energy to get started. This is called the activation energy (EA) given in kcal/mol. If the temperature gets higher, the acceleration of the reaction will increase. The reaction rate constant, k, quantifies the speed of the reaction. To be more specific, the reaction rate constant, k (as seen in appendix and eq. 2), is increasing according to higher temperature or by lowering the activation energy, E_A. The equation has shown to embrace empirically to a huge range of chemical reactions among those observed in food systems (Decker *et al*, 2010). This explains why Arrhenius equation is commonly applicable in accelerated shelf-life tests regarding food products.

The approximation aims to easily find the time and temperature correlation of oxidation rate. This is easily calculated by measuring the oxidation rate at three diverse temperatures at least. In a first order reaction, the activation energy (E_A) should result in a plot of the ln k and the reciprocal of temperature, 1/T, generating a slope (see appendix and eq. 10 for a mathematical example), as is illustrated in figure 3 below. A study performed by Giannakourou and Taoukis (2003) is a good example of a first order reaction, where the loss of the vitamin content in frozen vegetables under variable storage conditions are explained by an Arrhenius equation as a proper kinetic modeling method. In another kinetic study regarding the temperature dependency of primary- and secondary oxidation products in olive oil, was described using the Arrhenius equation (Gomes-Alonso *et al*, 2004). The data can be extrapolated further in order to find out the oxidation rate for a desirable temperature or to find the accelerating factor. The accuracy and a correct statistical approach of the extrapolated data remain although to be controlled. In addition, some deviations from proper linearity of Arrhenius equations can also be expected to be found at both accelerated and close to actual storage temperatures (Decker *et al*, 2010).

The E_A value, the activation energy, in a deterioration reaction is in a way made up. This since it is of course not really true that only one current chemical reaction is participating in the deterioration process. There are plenty of simultaneous processes going on at the same time causing the aging of foods. The E_A value for a certain reaction is though a valuable tool, since it brings on further information about the reaction process. The activation energy gives an indication about the fragility of the food and its deterioration process. Low activating energy indicates a faster break down. The E_A value will therefore give a hint when trying to predict further behavior of the food, e.g. predicting what will happen at different temperatures. Already observed E_A values of oxidation reactions are reported and could be found in the literature. Therefore no experimental studies are required. The oxidative indicators in the literature vary though to a large extent. In general oxidative rancidity reactions have a reaction activation energy of about 10-25 kcal/mol (see appendix and eq. 9 for a mathematical example) (Sewald and DeVries, 2003). As for example, an accelerated shelf-life estimation study of ripe olives at different temperatures was conducted by García-García *et al* (2008). This by using a first order reaction and an Arrhenius approach followed by Q_{10} application as discussed below. They predicted the shelf-life concerning color, firmness and pH.

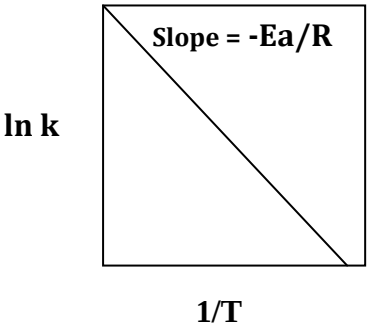

Figure 3: A typical Arrhenius plot were lnk are plotted against 1/T in order to get the slope of the line (Labuza and Fu, 1997)

5.5.2 Q_{10}- modeling

Accelerated shelf-life experiments are in general performed at different temperatures over actual storage conditions. What temperatures to be used are depending on what category of product to be analyzed. Usually, these kinds of accelerated storage temperatures are performed 10°C apart from each other, which explain the Q_{10}-modeling and the usefulness of generating a Q_{10}-factor. If the temperature unit °C is mentioned then the Q_{10} term is used in the literature. In case of the temperature unit Fahrenheit is mentioned, the expression q_{-10} is used in the literature (Labuza and Fu, 1997). The value of Q_{10} can be calculated from data of almost every kind of storage test, even if the temperatures are not 10 °C from each other. The study is supposed to be based on at least two different storage temperatures in order to generate a factor. The intention with Q_{10} modeling is to find a way to state the increase in the rate of reaction, when the temperature is increased by 10 °C. The Q_{10}- factor gives useful information about what to expect at different temperatures and due to that, a first indication of the fragility of the food (Sewald and DeVries, 2003).

In order to apply the equation for establishing the Q_{10}-factor, as seen in the appendix and eq. 4, the ratio of the rate constants is the inverse of the related shelf-life estimates observed at temperature T and T+10. The observed shelf-life values are mentioned and estimated in for example hours, days, or weeks and the ratio between these two estimates result into a Q_{10}-factor, as seen in appendix and eq.5 (Lawless and Heymann, 2010). The estimation of a Q_{10}-factor for a certain products will give a specific factor for the reaction rate according to the relationship between time and temperature. Q_{10}-value is therefore a measurement of the sensibility of the reaction´s dependence on temperature (Gibbons, 1979). The Q_{10} –value can be used when determining the accelerating effect instead of via the activation energy (Ragnarsson and Labuza, 1977).

The Q_{10}-value of 2 is usually found for enzymatically induced changes or degradation of pigments and flavors (Toledo, 2007). Products made of cereals suffering from enzymatic fat oxidation can therefore expect to demonstrate a Q_{10} value of about 2. Low fat cereals with no enzymatic activity have a Q_{10}-value of about 3. A small difference in the Q_{10}-value can influence the shelf-life prediction to a great extent (Sewald and DeVries, 2003). For example, the Q_{10}-value is 2 if the product is stored at 20°C with a shelf-life of

20 weeks and stored at 30°C with a shelf-life of 10 weeks (20 weeks/10 weeks). Q_{10} is defined as the reaction changes for every 10°C increase in temperature (Toledo, 2007). A Q_{10}-factor defined as 2, means that the reaction rate is duplicated when the temperature is elevated 10°C, from e.g. 20°C to 30°C. If a Q_{10}-factor is one, no change in reaction rate has occurred. The Q_{10}- value can also be calculated from the ratio between the rate constants at k_T and k_{T+10}, and not just through shelf-life estimation in weeks or days. This means in practice that the measurements, e.g. mentioned in mg/100g sample, can be used directly as seen in example and eq. 6 (see appendix). A shelf life estimation can be calculated even if the temperatures are not 10 degrees apart, see appendix and eq. 7 for example , were two temperatures are used 30°C apart (Lowe *et al*, 2001). A small difference in the Q_{10} of e.g. 0.5, can shift the shelf life prediction to a big extent (e.g. from 20 weeks at 20°C, to 32 weeks at a higher temperature, 12/20 weeks= 0,6 → a 60% difference in shelf-life prediction). If the Q_{10}- value is known, it is also possible to calculate the activation energy based on the same relationship. The Q_{10}-factor can easily be used further when approximating the activation energy, E_A, (see appendix and eq.8). The activation energy, E_A, is needed to calculate the accelerating factor.

5.5.3 Accelerating factor

Accelerated shelf-life tests are needed to find a proper acceleration factor, AF, for the tested product. There are mainly two possible procedures to enable this: Arrhenius equation and the Q_{10}-modelling method mentioned above. The E_A value can be obtained, either directly from the Arrhenius equation or according to the Q_{10}- modeling method mentioned above. The Arrhenius model might require several experimental values, at least three, in order to get a proper linear relationship. The Q_{10}-model on the other hand, requires at least two trials and is many times favorable since it is easier to perform. Both mathematical methods will result in a time and temperature relationship for a certain deterioration process, which will give the E_A, as well as the accelerating factor via further calculations. There are available databases offering activating energy data, E_A, for a many types of food systems. If proper information is found, no experimental work is required.

The E_A is significant information needed in order to determine the Accelerating factor (AF). The relationship regarding all parameters when deciding the AF at accelerated conditions and the applicable equation, is seen at eq. 11 (see appendix). The acceleration factor enables the conversion from accelerated temperatures back again to actual storage conditions at normal temperatures (Lawless and Heymann, 2010). According to a consumer acceptance and a shelf-life study performed by Hough et al. (2006), the activation energy, EA and the AF factor was of interest, when seeing where the sensory data correlated with the shelf-life of mince meat. The accelerating factor enables extrapolating of accelerated data back to normal storage conditions. The result is of course depending on many parameters, such as type of product, degradation mechanism, tendency to spoil, composition etc. Regarding the calculations, the temperature at accelerated conditions for oat-based products, the T_u is set to 8°C (281 K). Eight degrees is chosen due to the fact that it is slightly higher than normal recommended refrigerator temperatures. It might reflect some product abuse on a consumer point of view, where optimal storage conditions seldom are maintained properly. The accelerated temperature in the example is stated to 35°C, since this often is a proper temperature used when predicting oxidative reactions. As an example, the AF factor is calculated to a value of 6.5 (see appendix and eq.12) If presuming a failure

time of 45 days at an accelerated temperature of 35°, it is possible to find out the failure time, FT_u, at usage temperature and, T_u , at normal storage condition. This is done by multiplying the acceleration factor with the shelf-life estimates in days or weeks, e.g. at normal storage temperature at 8°C (see appendix and eq.13). The calculation hence results in a failure time of 293 days at normal storage temperature of 8°C (Lawless and Heymann, 2010).

6. RESULT

6.1 Oat drink production

The floating oil in water oat-based emulsion is quite simple itself. The secret is in the mixture of enzymes and the breakdown of starch giving the special texture. Oat flakes and water are simply mixed together and finally milled to a homogene suspension of an oat in water suspension (Agerbjer, 2007) and the enzymes are thereafter added. The enzyme preparation is really central for the process. The involved enzymes are responsible for the breakdown of starch and will let the fibers be kept intact. The enzymes are furthermore responsible for the sweetness and fullness of the product (Oatly AB, homepage, 2012). It is possible to easily remove most of the insoluble fibers and to keep the soluble ones in the water fraction. This is done in order to get a "milky texture". Some of the unsoluble fibers will on the other hand be discarded (Oatly AB, homepage, 2012). The oat-mixture is grayish in appearance and has a nutty taste of oat (Agerbjer, 2007). Decisively the product is homogenized in order to get a stable emulsion, a homogenous, smooth and plain product.

The products made from the ordinary oat-base have 0.5 % fat in the emulsion. Some of the products are also enriched with extra fat, up to about a 10% fat content. Flavored and fruity beverages made from Oatly's oat-base have a current shelf-life estimation set to five months, when quality changes are obtained. The largest quality losses regarding color and taste (rancidity due to secondary metabolites) are seen after 1-2 month, especially for flavored ones and the ones made of strawberries. The degree of quality failures declines after this period, which explains why the biggest changes are observed within this period. Today, the storage tests are conducted at direct conditions and are sensory tested by an internal test panel within the company. If the product seems to stand quality parameters, the study will be conducted at full-lengths. The test period lasts within a time range of about five months and even up to one year, depending on the outcome of the tests. This means in practice, that it may take up to 1.5 years before a new well-turned-out product can be launched on the market (Emelie Sellman, personal message, 2012).

6.1.1. Quality assurance

The hazardous critical control points (HACCP) and thereof the production of a safe oat-base is established out of the two main critical control points, the insurance of a tight package and a proper heat-treatment (Charlotte Grahn, personal message 2013). The products will undergo a UHT-treatment (140 degrees for 4-5 seconds, no fruit

beverages) or pasteurization (110 degrees for 4-5 second). The oat-based product is therefore primary packed in a proper aseptic package and thereafter heat-treated in order to destroy microorganisms to be sure that no contaminate will be able to spoil the product with an immediate connection to the production occasion. This is achieved to guarantee a safe product for the consumer, concerning microbial growth and contamination. Despite this action, there is always a possible risk for re-contamination, after heat-treatment during the production chain.

In order to achieve a high-quality product beside the guarantee of a safe product, the storage stability is in main focus. The most probable quality threat in oat-based product is due to rancidity development. To achieve as high quality as possible, the raw material has to be in accordance with the high-set quality specifications. The provider of the raw oat will thereof guarantee that the heat-treatment is proceeded according to conditions for a proper enzyme inactivation and microbiological standards (Charlotte Grahn, personal message 2013). The oat is therefore consequently heat-treated before it reaches the factory, which insures a totally inactivated enzyme activity (Emelie Sellman, personal message 2012). The microbiological standard includes specifications for microbiological growth, pesticide rests and toxins produced by fungi. These specifications of oat are therefore the two main objectives, in order to guarantee a high quality raw material able to process further to fulfill high set quality assurances (Charlotte Grahn, personal message 2013).

6.2 Potential accelerated shelf-life approach for oat-based products

Since the raw oat is heat-treated and ezymatically inactivated and the ready-made oat-base is pasteurized or UHT treated during process, it is most likely a sensory quality threat due to autooxidation over time. The main limiting shelf-life and quality parameter is therefore rancidity. The oxidation reaction is a first order degradation reaction, where the rate of change in the concentration is proportional to timescales. The detection and quantification of the main causing oxidative indicator, hexanal, is consequently of great interest. It is important to evaluate when the sensory perception correlates with the objective measurement of the total hexanal content in the sample. The definitions of the acceptability limit of the causing factor - the sensory “cut-off point” - is of greatest interest, at which level hexanal is perceived as unacceptable for consumers. Since Oatly´s oat-based oil-in-water emulsion products contain a low fat, the total hexanal content is the best oxidative indicator to measure.

6.2.2 Experimental design and mathematical implications

The main issue concerning shelf-life testing is though, that it will make no sense to test an oat-based product suffering from oxidative rancidity as the main quality failure over time at direct conditions. This is because of the fact that the process proceeds rather slowly. Therefore accelerated conditions are to prefer and applied for a faster predictive evaluation. This at least, in order to give a first hint about what to expect further at longer studies - if the study should be considered as totally ended or kept proceeded at full-time length. In order to apply a proper accelerated experimental approach, the chosen time and temperature applicable are of biggest significance. The experimental design mentioned beneath is based on the fact that no known activation energy and Q_{10}-factor is already known or considered as adoptable from the literature for oat-based products. This explains the need for a basic experimental study conducted from the

beginning, enabling the adoption of the activation energy, EA and out from that, a Q_{10}-factor. The best experimental conditions regarding sample amount, time and temperature are according to the literature defined as follows:

The test temperature should be conducted in a temperature range of between 20-60 degrees. A study conducted at a temperature over 50°C is of no interest, since the formations of hexanoic acid out of hexanal tend to occur at these higher temperatures (El-Magoli *et al*, 1980). Common test temperatures are therefore 10°C apart and conducted at for example 25, 35, 45 and 55°C. A sample used as a control, stored at 0°C, is recommended. The largest change is often observed in samples stored at lower temperatures, such as 25 and 35°C. Analysis each week is commonly performed and each testing temperature should have at least six data points over time to make the study results statistically reliable. At least two test temperatures are required for the study, but three or four is an ideal number for more precise shelf-life predictions (Sewald and DeVries, 2003*)*. Experimental studies conducted within 60 days period of time seem to generate good test results (Grosso *et al*, 2002, Lee *et al*, 2002, Trawatha *et al*, 1995).

The main task is consequently to find out information from accelerated kinetic models on such an easy basis as possible, with the intention of further conversion of correct, valuable data at actual storage conditions. This statement therefore calls attention to the Q_{10}-modeling approach, in front of the experimental design based of Arrhenius kinetics, both generating the thought after E_A for a specific reaction. The Q_{10}-modelling is favorable since there is no need for lots of test temperatures and for a plot generating the k-value and thereof the activation energy on a regression line. This Q_{10}-procedure gives a Q_{10}-factor and thereafter an E_A on a simple basis, which is further used in order to calculate and get to know the true effect of the important, calculated accelerating factor (AF). The AF factor specific for a certain reaction, will therefore enable the extrapolation of data back again, from accelerated studies to reverse information at normal storage conditions. This means in practice, calculations of number of weeks required at normal storage conditions it would take to lose the eating quality for a specific product.

The easiest, applicable accelerated approach to recommend, is therefore the shelf-life experiments due to Q_{10}-modeling, were two test temperatures and the correlating shelf-life estimates will generate a Q_{10}-factor as a result. This is in case there is not any activation energy already known from the literature or an already known Q_{10}-factor identified for the specific product of interest, worth considering to use. The chosen temperatures are often 10 degrees apart, where 25°C and 35°C as chosen test temperatures work as a suitable example, when dealing with auto oxidative reactions. The Q_{10}-factor based on shelf-life experiments is used further in order to calculate the E_A for the reaction of interest and out of the E_A the wanted AF factor is thereafter calculated thanks to available mathematical equations. The AF factor converts the accelerated shelf-life estimation results at 35°C, back to normal storage conditions at storage at 8°C. The assumption working as an example generated a shelf-life of 293 days.

7. DISCUSSION

The subject, shelf-life tests within quality assurance, is of much interest due to the fact that quality is always in main focus when dealing with food products. The complexity in a proper quality assurance within food production is the reality, when dealing with food products. There is not always a fast, clear and easy answer. Oatly AB is a good example, as the company has a big need to speed up their innovation program**.** As initially announced the largest quality changes according to taste and color of oat-based products have been observed in-house within two months period of time. This early shelf-life declaration of two month time showed out to agree well with the literature (60 days according to Grosso *et al*, 2002, Lee *et al*, 2002 and Trawatha *et al*, 1995). The gathered facts concerning sensory testing on an early basis also showed to correlate well with the literature found concerning a proper test procedure. The mathematical shelf-life estimations adopted working as examples concerning oxidative reactions showed out to agree well with values from the literature as well (concerning activation energy).

Since the raw oat product is heat-treated before further process, it ensures sterilization and enzyme inactivation. The most probable scenario is therefore a quality threat according to sensory spoilage due to rancidity through autooxidation. Bacterial and lipase contamination can though cause problems during storage since there is a risk for recontamination after production and during storage on a consumer point of view. The heat process of the raw oat may contribute to the destruction of the naturally occurring antioxidants in oats, e.g. the aventhramines. This heating-process will therefore add both pros and cons concerning the oxidative stability in oat. The limiting quality parameter in oat-based products, oxidative rancidity reaction, requires quite low activating energy, which hence explains why it occurs easily and frequently in stored products at normal temperatures. This happens particularly in open containers where oxygen is present to a high extent and especially in foods containing a high TGA content, such as in oat products. At higher temperatures, both oxidation and hydrolysis will occur to a higher extent. One question might be worth investigating further. Is it easier and cheaper to analyze color changes as a quality indicator, instead of an oxidative indicator? Another question concerning shelf life estimation of photosensitive foods under actual or accelerated conditions is if more parameters than time and temperature should be considered. There are some statements in the literature, where oxidative estimations cannot be correctly achieved, if the effect of light is not taken into account too. Both the effect of light and temperature according to time on oxidation rate might be important to take into consideration (Manzocco *et al*, 2012). This even if the effect of light probably is not the ruling factor in oat-based products.

The best oxidative indicator in low fat food, such as oat-based emulsions is, according to the literature, the total hexanal content. Hexanal is responsible for off-flavors and favorable to analyze since it is volatile and detectable at low levels. At temperatures above 50°C, hexanal will be converted into hexanoic acids. It is therefore no use testing such as products at higher temperatures. These facts explain the importance of choosing the right test temperature in relation to time when measuring the total hexanal content in a food sample. The objective measurement method of hexanal by using GC together with the Q_{10}- predictive method, is the given procedure concerning low fat foods. Q_{10}-modelling is favorable, since it is easier to perform in comparison to the Arrhenius model. A small difference in the Q_{10}- value can though have a great impact on

the shelf-life. One should also keep in mind that the reaction rate and the deterioration not always correlate as the temperature rises. As most sensory experts state, the analytical methods should be complemented by a sensory program. The chemical tests are many times less time-consuming and costly compared to sensory tests. A correlation between both physical and chemical tests will therefore increase the confidence of the sensory results (Labuza and Fu, 1997). One should though keep in mind that the best chemical quality is not always the same as that perceived as the best quality by the consumer (Gibbons, 1979).

8. CONCLUSION

There is most likely time and money to save with a proper accelerated study. This type of study will hopefully give a hint and point out the measureable, limiting parameter and the weakness of a product´s shelf-life on an early stage. There are however risks to consider, since it might be a general overconfidence within the food industry on accelerated storage tests according to its source of error and main purpose. The aim of this kind of test has to be clarified and accentuated, since the entire purpose is to give an initial hint of the weakness of the product. This initial data will give information whether the study should be ended on an early basis to save time and money, or to be continued at full-time length. An accelerated study cannot under any circumstances be the single deciding action to predict a product´s shelf-life. An accelerated shelf-life study should always be performed in parallel with a full-length study and together with sensory evaluation program. The sensory evaluation program should be considered as the major limiting parameter, due to the consumers' precipitation, acceptance and preferences.

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This master thesis gave me the opportunity and new insights and a way to look at, state and handle all including jigsaws within the concept- food science. I would like to once again give my warmest thanks to especially, Charlotte Grahn, product development manager and the former employee Emelie Sellman at Oatly AB, who first stated this problem in an open-minded-way without any prestige involved. The two of you gave me the idea and the important inspiring first input to really state this problem into an interesting and complex statement. This due to the fact, that it is a context of importance within the modern food industry - shelf-life and quality testing. Your need of my knowledge, skills and input in the subject - to have a look at it on a deeper level, gave me a great challenge to master.

I would like to aver, that sensory and mathematic kinetic models have not been well-covered and discussed during the agronomy program at the Swedish University of Agriculture Sciences. It has therefore been a big dare for me, to get a much deeper understanding and knowledge of each aspect and factor, worth taking in to account when dealing with sensory and quality assurance aspects. This, since it repeats already acquired knowledge gathered during my last five years of studies in addition to a few more dimensions taken into account, when focusing on a certain company and product area. It covers and sets together so many significant parts within the concept, food

science. This thesis therefore gave me, in my opinion, a high most reality-based problem with a great possibility to prove my personal skills as a master in food science. This by truly mastering the complex shelf-life and quality area in a reality-based-case-way of thinking.

10. APPENDIX

Calculations

$$\delta Q/\delta t = k(Q)^n \quad \textbf{(eq. 1)}$$

$$k = k_o e^{\left(\frac{-E_A}{RT}\right)} \quad \textbf{(eq. 2)}$$

$$\ln k = \ln k_0 \; - \frac{E_A}{R}\left(\frac{1}{T}\right) \quad \textbf{(eq. 3)}$$

k= A reaction rate constant to be estimated (e.g I_{ox} , Oxidative indicator)

k_o= A constant independent of temperature (often called frequency factor)

E_A= Activation energy (kcal/mol, specific for a certain reaction)

R= R is the molar gas constant (8.31 J/K/mol)

T= temperature (mentioned in Kelvin)

$$Q_{10} = \frac{k_{T+10}}{k_T} = \frac{S_T}{S_{T+10°C}} = ? \quad \textbf{(eq. 4)}$$

$k_T + k_{T+10}$ = Reaction Rate constants at temperature T + T+10°C

T and T+10 = Temperatures (T+10°C)

$S_T + S_{T+10}$ **=** Related shelf-life estimates at temperature T and T+10 (e.g. 20 weeks/10 weeks)

$$Q_{10} = \frac{S_T}{S_{T+10°C}} = \frac{\mathbf{S_{10°C}}}{\mathbf{S_{20°C}}} = \frac{\mathbf{20}}{\mathbf{10}} = \mathbf{2} \quad \textbf{(eq. 5)}$$

$$Q_{10} = \frac{k_{20°C}}{k_{10°C}} \frac{400 \text{ mg } /100 \text{ g Hexanal content}}{200 \text{ mg}/100 \text{ g Hexanal content}} = \mathbf{2} \qquad \textbf{(eq. 6)}$$

$$S_{T2?} = \frac{S_{T1}}{Q_{10}^{\left(\frac{T_2-T_1}{10}\right)}} = \qquad \textbf{(eq. 7)}$$

S_{T2} = ?

S_{T1} = 20 weeks

T_1= 25°C

T_2= 55°C

$$S_{55°C} = \frac{S_{T1}}{Q_{10}^{\left(\frac{T_2-T_1}{10}\right)}} = \frac{20}{\frac{30}{10}} = \frac{20}{(2)^3} = \mathbf{2,5 \text{ weeks}}$$

$$\ln Q_{10} = \frac{10E_A}{RT(T+10)} \qquad \textbf{(eq. 8)}$$

T= temperature (mentioned in Kelvin, 273+8°C = 281, 273+35°C= 308)

R= R is the molar gas constant (8.31 J/K/mol)

$$\ln 2 = \frac{10E_A}{8.31*281*308} \rightarrow$$

$$\ln 2 = \frac{10E_A}{719213{,}88 \text{ J/mol}} \rightarrow$$

$$719213{,}88 \text{ J/mol} * 0{,}6931471806 = \frac{10E_A}{656519{,}02 \text{ J/mol}} * 656519{,}02 \text{ J/mol} \rightarrow$$

$$\frac{498521{,}0731 \, J/mol}{10} = \frac{10E_A}{10} \rightarrow$$

$$E_A = \frac{498521{,}0731 \, J/mol}{10} = \mathbf{49852{,}10731 \text{ J/mol} \rightarrow 49{,}85210731 \text{ kJ/mol} \sim 50 \text{ kj/mol}}$$

1 kcal/mol = 4,184 kJ/mol → **(eq. 9)**

$$E_A = \frac{49{,}85210731}{4.184} = 11{,}91493961 \sim \mathbf{12 \text{ kcal/mol}}$$

$\frac{E_A}{R} \rightarrow \frac{49852{,}10731\ \text{J/mol}}{8.31\ \text{J/K}*\text{mol}} = 5999{,}050218 \sim \mathbf{5999}$ **(eq. 10)**

AF (if known E_A) = **(eq. 11)**

$$\text{AF} = \exp\left[\frac{E_A}{R}\left(\frac{1}{T_u} - \frac{1}{T_{test}}\right)\right]$$

AF= Accelerating factor

$exp\ (x) = \ e^x$

$T_u = actual\ storage\ temperatur$ (8°C, fridge)

$T_{test} = Accelerated\ test\ temperature$ (35°C)

$$AF = exp\left[5999\ \left(\frac{1}{281} - \frac{1}{308}\right)\right]$$ **(eq. 12)**

$AF = exp\ [5999\ * 3{,}119656145e^{-4}]$

$AF = e^{1{,}871481721}$

AF= 6,497917371~ 6.5

AF= **6.5**

(FT= failure time) **(eq. 13)**

$FT_{35°C}$= FT_{test}* (AF)= 45 days*(6,5)= 292,5 ~ **293 days**

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