CHALMERS

Use of Vegetable Oils as Processing Aid in Dry Spice Mixtures

Effect on Rancidity, Separation, Dusting and Caking

Master of Science Thesis in the Master Degree Program Biotechnology

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Abstract

Santa Maria AB is the leading seasoning company on the Nordic market. Spice mixtures are the basis for several products among the five flavouring product concepts; Tex Mex, Thai, India, Barbeque and Spices. During the manufacturing process of spice mixtures, palm oil is used as processing aid in order to reduce dusting and caking, and to make the seasoning more homogenous by preventing separation of particles of different sizes. Due to health concerns and environmental aspects, Santa Maria AB aims to reduce the use of palm oil. The purpose of this Master's Thesis is to substitute the palm oil used as processing aid in the manufacturing of spice mixtures produced at Santa Maria AB, Mölndal, for a vegetable oil that promote health and that is produced under environmental-friendly and sustainable conditions.

The stability of ten different vegetable oils was investigated using accelerated shelf life tests evaluated by sensory analysis. All oils were stable enough not to deteriorate during storage for twelve weeks at room temperature and 31°C. The intensity of rancidity perceived during storage at 40°C and 60°C was plotted versus time, in order to retain an Arrhenius plot to determine the temperature dependence for each oil.

The results showed that the oil included in the spice mixtures does not have to be as stable as an oil intended for high temperatures, for example for frying. This is mainly due to that the seasonings are stored in a dry place, not above room temperature, and that it is unlikely that the spice mixtures will become rancid during the expected storage time. Rapeseed oil and sunflower oil were selected for further analysis to investigate their effect on separation, dusting and caking of spice mixtures, compared to palm oil and samples without addition of oil. These parameters were evaluated by visual analysis and measurements of angle of repose. The results showed that addition of oil as processing aid is important to maintain spice mixtures of high quality. However, the type of oil did not affect the results.

The recommendation to Santa Maria AB is to replace the palm oil used as processing aid in dry spice mixtures for Swedish cultivated rapeseed oil. This would result in several advantages, such as reduction of the environmental impact, healthier products and reduced costs.

Keywords
Accelerated shelf life testing, Angle of repose, Caking, Dusting, Oxidation, Processing aid, Rancidity, Sensory evaluation, Separation, Spice mixture, Vegetable oil

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1 Introduction

Santa Maria AB started as a small spice shop in Gothenburg in the beginning of the twentieth century and is today the leading flavouring company in the Nordic region. The company, with more than 1400 employees, is a part of the Finnish food corporation Paulig Group. Santa Maria AB has developed several flavouring product concepts, such as Tex Mex, Thai, India, Barbeque and Spices, which are sold in more than 30 European countries. The spice selection consists of almost 100 different spices which make the basis for all seasoning products of the remaining food concepts. The production of spices and spice mixtures is located in Mölndal, Sweden and Saue, Estonia, while the Mexican range of products are manufactured in additional production sites in Sweden, England and Belgium (Santa Maria AB, 2012).

When producing spice mixtures, addition of oil is necessary as processing aid. The oil has a significant impact on the properties of spice mixtures, especially to reduce dusting and caking and to prevent separation of particles of different sizes (Kjellmer, 2012). Today fractionated palm oil, palm olein, is used as this processing aid, as it has the appropriate properties and is a stable oil (Bringsarve, 2012). When discussing the palm oil used in the spice mixtures, it is the palm olein fraction that is referred to.

Compared with other oil crops, the oil palm gives a very high yield per hectare. This has led to a rapidly expanding world industry of oil production in the tropical areas of Asia, Africa and America, especially Malaysia and Indonesia (Corley & Tinker, 2003; WWF UK, 2011). As the request for palm oil increases, new plantations develop to meet the demand. This causes conversion of large areas of tropical forests with high biological diversity, leading to threat of extinction for several species. The last twenty years, the area for oil palm cultivation has increased almost eightfold, and the demand is expected to double again by 2020 (WWF, 2010). To promote the growth and use of sustainable palm oil, the organisation Roundtable of Sustainable Palm Oil (RSPO) was formed in 2004 by the initiative of World Wide Fund for Nature (WWF). RSPO has developed and supports global environmental, social and economic viable principles and criteria in the oil palm industry, in order to supply the world with enough sustainable palm oil without harming the planet and its people (RSPO, 2009; WWF, 2010). Santa Maria AB is one of the supporting members of RSPO (RSPO, 2009; Santa Maria AB, 2012).

Santa Maria AB aims to reduce the use of palm oil partly due to environmental aspects, but also of health concerns, since palm oil consists mainly of saturated fatty acids. The company is working to replace the palm oil in the Mexican food selection, in products containing high amounts of oil. Therefore it is natural to take the step to replace the palm oil also in the manufacturing process of spice mixtures.

1.1 Purpose and Objective

The purpose of this Master's Thesis is to replace the palm oil used as processing aid in the manufacturing of spice mixtures produced at Santa Maria AB, Mölndal, for a vegetable oil that promote health and that is produced under environmental-friendly and sustainable conditions.

It is important that the oil fulfills several demands to be suitable as a processing aid, including stability during the shelf life, and inhibition of separation, dusting and caking of spice mixtures during manufacturing. Appropriate methods to control these parameters are to be elaborated, as well as evaluation of practical experiments. This is done by investigation of a number of possible alternative vegetable oils, and how these different oils affect dusting, separation, agglomeration and caking in four chosen spice mixtures. The stability and rancidity of the vegetable oils will be examined using accelerated shelf life testing, and evaluated by sensory analysis. In order to evaluate caking and dusting of spice mixtures a method of measuring the angle of repose of seasonings will be conducted.

Another important aspect to consider when choosing new oil as processing aid is to avoid increasing costs.

1.2 Limitations

This Master's Thesis will focus on the parameters taste, appearance, rancidity, dusting, separation and caking of spice mixtures when possible alternative vegetable oils as a replacement for palm oil are to be investigated. Other parameters will not be studied.

A limited number of oils will be tested, whereas all will be of vegetable origin. By practical reasons, the tests will be performed using only four of the spice mixtures containing palm oil today. Due to limited time, accelerated tests will be performed and not real time trials.

The stability and rancidity of the chosen vegetable oils will be evaluated by sensory analysis performed by a panel at Santa Maria AB. This evaluation will be performed as a screening test and not a complete sensory profiling. Some samples are to be analysed also by the analytical measurements peroxide value and *para*-anisidine value. Other evaluation methods, such as investigation of which flavour compounds that are formed during the deterioration, will not be performed. The properties of dusting, caking and separation will be studied using only the method angle of repose, and visual analysis.

2 Theoretical Background

The following parts will cover the theoretical background of the project, divided in the areas Spices and Spice Mixtures, Shelf Life of Foods, Vegetable Oils and Lipid Oxidation.

2.1 Spices and Spice Mixtures

Spices are used to stimulate the appetite and to add flavour, colour and texture to food. The word spice originates from the Latin word *species*, meaning specific kind. This refers to that all parts of a plant, including the seed, berry, leaf, stem or root, may be used to add aroma, fragrance or pungency (Raghavan, 2000).

Spices are not only used as flavourings and colourings, but have for long been known for their preservative and health properties as antimicrobials and antioxidants (Kalemba & Wajs, 2011; Takemasa & Hirasa, 1998). Even though spices constitute a very small part of a meal, they may make an important contribution to our daily antioxidant intake. The antioxidant capacity of spices is thought to be the result of the presence of phenolic compounds (Lu et al., 2011). Currently, rosemary and sage are used as natural antioxidant additives in foods (Reische et al., 2008).

Spices are used in several different forms; fresh or dried, whole, crushed, ground or powdered (Kalemba & Wajs, 2011; Raghavan, 2000). Using dried spices entails advantages such as easy handling, longer shelf life and higher taste intensity compared to fresh ones. However, the aroma of dried spices is less than for fresh, and the flavours can be oxidised resulting in losses during milling and storage (Raghavan, 2000).

Spices provide a wide range of different tastes to foods, and by combining different spices and other flavourings in a spice mixture or seasoning, a vast spectrum of flavours can be created (Raghavan, 2000; Takemasa & Hirasa, 1998). The usage of ready-to-use spice blends are becoming more and more popular since these kinds of products are easy to use, saves time in the kitchen and gives the possibility of experiencing new flavours from different parts of the world (Kalemba & Wajs, 2011).

Spice mixtures are an important part of the wide variety of products of Santa Maria AB and are available in all five flavouring product concepts; Tex Mex, Thai, India, Barbeque and Spices. Santa Maria AB works a lot with improving existing products and the company focus strongly on new concepts and trends when developing new products. A core in the developmental process is to create high quality products with irresistible taste sensations, making the control of the manufacturing process of spice mixtures important.

2.1.1 Manufacturing Process of Spice Mixtures

The raw materials for the spice mixtures produced at Santa Maria AB are grown all over the world. The spices are washed, dried, peeled, crushed or grinded in different fractions in order to suit as components in different products. This is made either at the spice factory of Santa Maria AB or by the suppliers. All raw materials are controlled for quality testing of flavour and appearance and absence of microorganisms before blending and packaging. As a safety precaution and to protect the spices from microorganisms, most ingredients are heat treated by hot steam (Snickert, 2012). The seasonings are mixed in a large blender and the ingredients are added one by one. When all dry ingredients are added, the mixture is released to the

blender where liquid ingredients, such as oleoresin and the processing aid palm oil are added. The spice mixture is blended for ten minutes before it is filled in large sacks, in order to be packed in the appropriate consumer packaging. The palm oil used in the spice mixtures has a melting point of 24°C and therefore it has to be heated prior addition. This is made by storing the container with oil on a heat plate (Blomstrand, 2012).

2.1.2 Food Powders

Spices and salt are examples of food powders, which are defined as dried and comminuted ingredients of biological origin consumed by humans and animals (Cuq et al., 2011; Fitzpatrick & Ahrné, 2005). Production of food in powdered form prolongs the shelf life of the product by reducing the water content and thereby reducing the degradation and deterioration of the product. The advantage of using food in powdered form is consequently to maintain the stability of the product during time, from production until it is to be utilised (Fitzpatrick & Ahrné, 2005). The low water activity ensures physiochemical and microbiological stability, preserving the safety and functionality of the food powder until use. Water elimination also reduces the total mass of the product, reducing the costs for transport (Cuq et al., 2011).

Food powders have many industrial applications but are also marketed directly to the consumers, making food powders present in everyday life and appreciated for being easy to store and use. Typically, food powders are not consumed in the powdered form but in a wet formulation, mixed with water and other added ingredients to the actual product being consumed (Cuq et al., 2011; Fitzpatrick & Ahrné, 2005).

2.1.3 Separation and Dusting

Mixtures of different food powders containing several ingredients of different particle properties and sizes can be segregated or separated after the mixing process. This problem can be limiting for the creation of food powder mixtures. Handling of food powders may cause dust generation, which can lead to health problems and allergy, or contamination due to dust settling and sticking onto equipment. Therefore prevention and control of dusting is important when handling food powders (Fitzpatrick & Ahrné, 2005).

The cohesiveness, and thereby also the dustiness of a powder, is determined by the particle size, particle shape, electrostatic charge and surface properties of the powder. A powder may release dust particles in many repeated pourings and not necessarily in a single handling (Wells & Alexander, 1978).

2.1.4 Caking Phenomena and Flowability

The agricultural origin of food powders results in a natural variability of the technological behaviour of food powders, due to variations in species and varieties, seasonal effects and differences in growth conditions and location. Food powders consist of chemically complex molecules containing mainly carbon, hydrogen, oxygen and nitrogen atoms. These molecules have the capacity to form different interactions, such as hydrogen bonds, hydrophobic interactions, ionic interactions and disulphide bonds. These different interactions contribute to the technological behaviour of a food powder such as hydration properties and the contact mechanism between particles, such as adhesion, agglomeration, caking and flowability (Cuq et al., 2011).

Caking is a continuous and complex, time-dependent phenomenon where a low-moisture, free-flowing powder adheres to form clumps and eventually large agglomerates. It is a deleterious problem in the food industry and results in loss of functionality and lowered quality of the food powder. Caking contains several stages, and in the beginning of the process inter-particle bridges start to form as a result of surface deformation. These interactions may disintegrate under mild shaking, conversely to the particle clumps formed during the later stages of agglomeration, having structural integrity. During the caking process, clumps of different sizes and varying degree of hardness may be present. Caking depend both on internal factors, such as particle size, charge and hygroscopic behaviour, as well as external factors such as relative humidity, temperature, applied mechanical stress and the presence of moisture barriers (Aguilera et al., 1995).

Powders consisting of large particles have better flowability compared to powders with small particles. The cohesiveness increases as the particle size decreases, making small particle powders possible to cause handling problems (Janjatović et al., 2011). The cohesiveness of a powder also increases with increased water content, and high moisture content affects its flowability and caking properties negatively (Aguilera et al., 1995; Janjatović et al., 2011).

A dry powder can absorb water from the moist air in the headspace, or through the package material or seal, when it is packed. The extent of absorption depends on the powder components' capability to establish interactions with water (Aguilera et al., 1995; Cuq et al., 2011). Ingredients with hydrophilic properties cause the hygroscopic character of food powders, which can lead to caking problems during storage. Some examples of these kinds of components are sugars, polysaccharides, protein hydrolysates, salts, powdered vegetables and yeast and meat extracts (Aguilera et al., 1995; Cuq et al., 2011; Hartmann & Palzer, 2011).

To minimise the caking of food powders it is important to have a strict control of moisture content and to store the products at low temperatures. In order to improve the flowability and inhibit caking, anti-caking agents can be added to hygroscopic food powders. The anti-caking mechanism is achieved by several different actions, for example by competing with the host powder for available moisture, by acting as a physical surface barrier between particles, or by forming a moisture-protective barrier on the surface of otherwise hygroscopic particles. Many spice mixtures produced at Santa Maria AB contain the anti-caking agent silicon dioxide that acts as a physical surface barrier between particles by interfering with the liquid bridging mechanism (Aguilera et al., 1995).

2.1.5 Methods to Evaluate Caking

Some methods used to characterise caking phenomena are flowability, cohesion, caking index and angle of repose (Aguilera et al., 1995). The angle of repose is a well-known powder property and one of the most important parameters to characterise the behaviour of granular materials (Geldart et al., 2006; Zhou et al., 2002).

There are several methods of measuring the angle of repose and due to the different values maintained from each method, published values of the angle of repose are seldom comparable. The two most common techniques are based on pouring the powder into a funnel, either held at a fixed height above a flat base, or raised gradually to allow the sample to flow out, as visualised in figure 2.1. Commonly, angles of repose below 30° indicate good flowability, 30°- 45° some cohesiveness, 45°- 55° true cohesiveness, and above 55° very high cohesiveness and very limited flowability. A more general classification is based on an angle of repose of 40°, where values below 40° indicate a free-flowing powder and above 40° a cohesive powder (Geldart et

al., 2006). The angle of repose decreases with increased particle size and increases with increasing friction coefficients, as well as if the particles deviate from the shape of a sphere (Zhou et al., 2002).

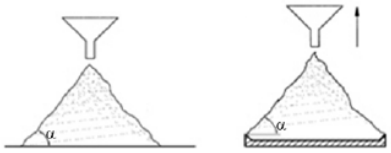

Figure 2.1: Measurement of angle of repose, *α*, using a fixed height (left) and fixed base (right). (Adapted for use from Geldart et al., 2006.)

2.1.6 Ingredients

The spice mixtures produced at Santa Maria AB contain a wide range of different ingredients, such as salt, sugars, spices, herbs, aromas, extracts, bouillons and other functional components. They all behave differently regarding stability and shelf life. The most commonly occurring ingredients in the four spice mixtures used in this thesis are described below. Several of these ingredients have hygroscopic properties which may cause agglomeration and caking.

Salt

Salt, NaCl, is a major component of many spice mixtures added to improve the flavour of the product. It also has preservative and antimicrobial capacity by reducing the water activity of the product (Coultate, 2009). Additionally, salt can be used as a carrying agent for dry ingredients (Igoe, 2011). Salts have hygroscopic behaviour and have the ability to interact with other components in the food, affecting the hydrophobic/hydrophilic interactions between molecules (Aguilera et al., 1995; Calligaris & Nicoli, 2006; Cuq et al., 2011). Contradictory literature data has existed whether NaCl would have a pro-oxidative or antioxidative effect, but it is concluded that NaCl has no effect on lipid oxidation (Calligaris & Nicoli, 2006).

Sugar

To add sweetness and to improve the flavour of seasonings, sugars such as sucrose or dextrose are used. Ordinary sugar, the disaccharide sucrose, consists of the monosaccharides glucose and fructose and is obtained from cane or beet sugar. Dextrose, also named glucose, is a reducing sugar commercially produced by enzymatic hydrolysis of starch (Igoe, 2011). The perception of sweetness is somewhat less for glucose compared to sucrose (Coultate, 2009). Sugars can be granulated or powdered, and are hydrophilic, causing hygroscopic properties (Aguilera et al., 1995; Cuq et al., 2011).

Garlic and Onion

Garlic, *Allium sativum*, and onion, *Allium cepa*, are widely used to provide a characteristic pungent flavour to several types of food. Both onion and garlic can be used fresh or dried, and the dehydrated forms can be chopped, granulated or powdered (Kalemba & Wajs, 2011; Raghavan, 2000). The flavour of onion and garlic ranges from mild and sweet to strongly pungent depending on the variety and origin, and becomes sweeter when cooked. The sulfuric

flavour compounds are formed enzymatically only when the cells are cut or damaged (Raghavan, 2000) and in dehydrated form the flavour enzyme is released only in contact with water (Igoe, 2011). Garlic and onion powder are highly hygroscopic (Hartmann & Palzer, 2011).

Chili and Paprika

There are five species of the genus *Capsicum*, whereas most pungent chili peppers and the more sweet paprika belong to the species *Capsicum annum*. The chili peppers have different size, shape, colour, flavour and degree of pungency (Raghavan, 2000). The pungency evolves from a group of molecules called capsaicinoids with capsaicin having the most pungent flavour (Coultate, 2009; Raghavan, 2000). The colouring components of chili and paprika are carotenoids, with capsanthin being the most common one (Coultate, 2009; Takemasa & Hirasa, 1998). These carotenoids are sensitive to degradation through oxidation catalysed by light and high temperature, causing loss of colour of paprika powder during storage (Raghavan, 2000). Paprika oleoresin is an extract isolated from paprika used as a colouring agent in several food products, being more stable to heat and light (Kalemba & Wajs, 2011; Raghavan, 2000). Paprika extract and dried paprika and chili in powdered or chopped form are used to add both flavour and colour to many spice mixtures produced by Santa Maria AB.

Tomato

Tomato is used both as a powder and dried in pieces as a component in spice mixtures. The red colour of tomatoes originates from the carotenoid lycopene, being relatively resistant to thermal degradation. However, the processing conditions to manufacture tomato powder cause degradation of lycopene, as well as oxidation, leading to loss of red colour during storage. Tomato powder has hygroscopic properties, leading to increased cohesiveness and caking problems during storage (Liu et al., 2010).

Pepper

Different varieties of pepper are important components in spice mixtures, giving aroma and pungency to the products. Black, white and green peppers are the berries from the plant *Piper nigrum*, picked at different stages of growth and processed at different ways. Green pepper is picked when the berries are unripe, whereas black and white peppers are ripe berries with the difference that white peppercorns are peeled before drying (Raghavan, 2000). The aroma, flavour and pungency vary between the different peppers, depending not only on variety but also on growth location (Kalemba & Wajs, 2011; Raghavan, 2000). Peppers are used ground in coarse or fine fractions in several of the spice mixtures of Santa Maria AB.

Herbs

Different dried herbs are used either crushed or ground in the majority of the spice mixtures, to add herbaceous and aromatic flavours to the products. Oregano, parsley, thyme, basil, sage and tarragon are components of the four seasonings included in this project. Other herbs frequently used in Santa Maria products are coriander, marjoram, dill and rosemary. Each herb has its specific aroma and flavour, giving each of these spices its own characteristics (Kalemba & Wajs, 2011; Raghavan, 2000). Spices from the *Labiatae* family, including rosemary, sage, oregano, basil and thyme, has shown to possess very high antioxidant capacity and high levels of phenolics, whereas spices in the family *Umbelliferae*, such as dill, coriander, and parsley, have low antioxidant capacity (Lu et al., 2011).

Anti-caking Agent

The anti-caking agent silicon dioxide is added to many of the spice mixtures produced by Santa Maria AB in order to ensure better flowability and to prevent agglomeration and caking of the dried ingredients (Igoe, 2011; Raghavan, 2000). Silicon dioxide has the ability to absorb approximately 120 % of its weight still remaining free flowing, and is added to many food products to prevent caking caused by moisture (Igoe, 2011).

Oil

Palm oil is added to the spice mixtures as a processing aid to make the blend more homogenous in order to prevent separation of particles of different sizes, and to reduce dusting and caking during manufacturing and packaging (Kjellmer, 2012). Processing aids like these, not having a function in the finished product and which will not cause a hypersensitivity reaction does not have to be declared as an ingredient (Livsmedelsföretagen Li, 2006).

2.2 Shelf Life of Foods

Handling, processing and storage of foods lead to alterations and deterioration of the food product over time. Several chemical and biochemical reactions, for example oxidation and hydrolysis, cause alteration of quality attributes such as texture, flavour and colour of the food. The nutritive value and the occurrence of toxic substances will also be influenced (Fennema et al., 2008). A number of simultaneous processes like these, often with complex kinetics, result in the deterioration of a food during storage (Hough, 2010).

One of the most significant quality deteriorating factors indicating the end of shelf life of a food product is lipid oxidation. Oxidation of lipids occurs when oxidation products react with several different kinds of other constituents in the food product, and alters the texture, flavour, colour and nutritive value of the food. There is also a possibility for toxic substances to be generated (Fennema et al., 2008).

The shelf life of a food product is defined as the time the product will remain safe, preserve the desired sensory, chemical, physical and microbiological characteristics and maintain the nutritional values declared, when it is stored under the recommended conditions (Kilcast & Subramaniam, 2000).

2.2.1 Packaging

In order to preserve a food product and maximise or extent its shelf life, correct packaging of food products are important. To avoid both biotic and abiotic spoilage of a food product the packaging has to make a barrier to moisture, oxygen and light. For products such as herbs and spices it is also desirable to prevent loss of volatile substances, in order to preserve the aroma and flavour of the product and inhibit contamination of adjacent products. The packaging is intended to deliver safe and attractive products in a convenient way to the market, as well as providing information to the costumer and selling the product. These requirements must be fulfilled at a tolerable economical level and within environmental acceptability (Emblem, 2000).

The most used packaging materials for herbs and spice products produced for retail consumers at Santa Maria AB are glass jars and laminate bags. The jars are made of

transparent glass making a total barrier to moisture and oxygen, while the lid made of plastic not composes a complete barrier. The laminate bags have a protective barrier made of polyethylene (PE) and ethylene vinyl alcohol (EVOH) in three layers, PE/EVOH/PE. The PE layers protect against moisture while EVOH makes an oxygen barrier (Yhlen, 2012). Generally the spice mixtures intended to be consumed all at once are sold in laminate bags, while products being consumed during a longer period of time are packed in jars, to make the product as convenient as possible for the consumer (Zetterquist, 2012).

The spices and spice mixtures produced by Santa Maria AB have a shelf life of between one and three years, in conformity with the guidelines conducted by Livsmedelsföretagen Li (2006). This relatively long shelf life is possible as a result of spices being regarded as non-perishable, only having fading colour and fading flavour intensity during storage (Zetterquist, 2012).

2.2.2 Accelerated Shelf Life Testing

To establish the shelf life of a food product, accelerated storage tests can be made to considerably shorten the time needed to obtain enough experimental data. This is of great importance especially when the predicted shelf life of the food product is expected to be long. Accelerated shelf life testing (ASLT) refers to any method evaluating the long-term stability of a product based on short-term tests (Hough, 2010; Mizrahi, 2000). The principle of ASLT is to expose the food product to a selected abuse condition, such as temperature, humidity or light at high levels, evaluate its quality commonly by sensory analysis, and extrapolate the results to normal storage conditions (Hough, 2010; Taoukis & Labuza 1996). Compared to storage during normal conditions, this test procedure saves both time and money (Hough, 2010).

By a systematic study of the deterioration process of a food product it is possible to determine the shelf life of the food product and ASLT is applicable to any deterioration process with a valid kinetic model. The process may be chemical, physical, biochemical or microbial (Mizrahi, 2000; Taoukis & Labuza, 1996).

Change of food quality can be characterised either by the loss of a quantifiable quality attribute A, or by the formation of an undesirable attribute B. The rate of degradation of A, for example a nutrient or a flavour, and formation of B, for example an off-flavour or discolouration, can be expressed by equations 2.1 and 2.2, respectively.

$$-\frac{d[A]}{dt} = k[A]^n \tag{2.1}$$

$$\frac{d[B]}{dt} = k'[B]^{n'} \tag{2.2}$$

The constants k and k' are the reaction rate constants and n and n' represents the order of reaction (Taoukis & Labuza, 1996). Equations 2.1 or 2.2 can be integrated to express A or B as a linear function of time t, as in equation 2.3.

$$F(A) = kt \tag{2.3}$$

The function $F(A)$ is defined as the quality function of the food and is expressed depending on the order of the reaction (Taoukis & Labuza, 1996). Note that A is the attribute which can be described as a function of time.

Most chemical or microbial reactions causing shelf life loss follow zero order or first order kinetics. A reaction is following zero order kinetics if the concentration plotted versus time results in a straight line, that is when $F(A) = A_0 - A$. A first order reaction obtains a linear relationship when logarithmic coordinates of the concentration is plotted against time, when $F(A) = \ln A_0 - \ln A$. If the inverse concentration is plotted versus time and a straight line is obtained, the reaction follow second order kinetics and $F(A) = \frac{1}{A_0} - \frac{1}{A}$. This denotes that the order of a reaction can be defined based on a few measurements by the plot maintaining the best linear relationship, and the quality function of the food, $F(A)$, can be determined (Taoukis & Labuza, 1996).

There are several environmental factors influencing the shelf life of a food product, for example temperature, relative humidity, light and mechanical stresses. The factor most commonly used to accelerate reaction rates and to incorporate in shelf life models is temperature (Taoukis & Labuza, 1996).

2.2.3 Arrhenius Model

One model widely used to relate the rate of a chemical reaction to temperature is the Arrhenius relation, denoted in equation 2.4.

$$k = k_A \, exp\left(-\frac{E_A}{RT}\right) \tag{2.4}$$

The constant k represents the reaction rate constant (kJ/mol K), k_A denotes the Arrhenius equation constant, E_A is the activation energy (kJ/mol), T is the absolute temperature (K) and R represents the universal gas constant (8.3144 J/mol K) (Taoukis & Labuza, 1996).

To estimate the temperature effect on the reaction rate of a specific change of food quality, values of k are estimated at different temperatures and the natural logarithm $\ln k$ is plotted versus inverse temperature $1/T$. This obtains a straight line with the slope $-E_A/R$, from which it is possible to calculate the activation energy. The activation energy represents the barrier of energy to overcome in order for the reaction to occur. The Arrhenius approach enables collection of data at high temperatures, during relatively short time, to extrapolate for the reaction rate constant at lower temperatures. To ensure the confidence limits for the Arrhenius parameters are narrow, an optimum number of five or six experimental temperatures are required. If only three experimental temperatures is used it is possible to use statistical methods to increase the accuracy (Taoukis & Labuza, 1996).

Using too high temperatures when conducting ASLT experiments may induce changes of a food product which would not occur during normal storage conditions, such as formation of other deterioration products or change of phase (Hough, 2010).

2.3 Vegetable Oils

Lipids are a diverse group of chemical compounds, associated with living systems, which are soluble in organic non-polar solvents (Coultate, 2009). The most common lipids of plant and animal origin are glycerides, and the major constituent of oils and fats are triglycerides. Other lipid components present in fats and oils are monoglycerides, diglycerides, free fatty acids, phospholipids, sphingolipids, sterols, waxes, vitamins and pigments, remaining from the cell membranes from which the oil is extracted (AAK AB, 2007; Arvanitoyannis et al., 2009).

The difference between oils and fats is that fats are solid and oils are liquid at room temperature. However, all vegetable oils in this Master's Thesis will be referred to as oils, irrespective the melting temperature. Oils and fats consist of a mixture of triglycerides, one ester derived from glycerol and three fatty acids (Coultate, 2009). A fatty acid is a mono-carboxylic acid with an unbranched aliphatic chain, which consist of an even number of between 4 and 28 carbon atoms (Arvanitoyannis et al., 2009; Coultate, 2009). The chain can be saturated, containing only single bonds, or unsaturated with one, two or up to six double bonds in *cis* configuration. The shape of a saturated hydrocarbon chain is straight, and insertion of a *cis* double bond introduces a bend of about 42° to the chain, significantly changing the shape and properties of the molecule (Coultate, 2009). The structure and melting point of the most commonly occurring fatty acids in natural fats and oils can be seen in table 2.1.

Table 2.1: The structure and melting point of the most common fatty acids present in vegetable fats and oils (Coultate, 2009).

Fatty acid	**Structure**	**Melting point** [°C]
Lauric	C12:0	44.2
Myristic	C14:0	54.1
Palmitic	C16:0	62.7
Stearic	C18:0	69.6
Oleic	C18:1	10.5
Linoleic	C18:2	-5.0
Linolenic	C18:3	-11.0

Natural fats and oils consist of a mixture of simple triglycerides, with three identical fatty acids, and mixed triglycerides, containing more than one type of fatty acid residues. Since the melting point of triglycerides depends on the unsaturation and length of the hydrocarbon chains, and natural fats are mixtures of different triglycerides, a fat has a melting range rather than a discrete melting point (Coultate, 2009).

The oils extracted from plants in nature have a wide range of fatty acid composition. Plants grown in cold and tempered climates generally contain oil which is liquid in room temperature, while a tropical climate yields fats with higher melting points (AAK AB, 2007). Due to variations in growth conditions, the fatty acid composition of the same oil or fat can vary slightly (Coultate, 2009). In table 2.2, the fatty acid compositions of the vegetable oils used in this project are presented, according to product specifications. These compositions are also confirmed by Gunstone (2008). The fatty acid composition divided in saturated (SFA),

monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids are given in table 2.3, for an easy comparison.

Table 2.2: Fatty acid composition of vegetable oils ([1]AAK AB, 2007; [2]Product specifications AAK AB, 2012).

Vegetable oil	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
Coconut oil[1]	8	6	47	18	8	2	6	2	
Palm olein[1]				1	39	4	43	11	
Rapeseed oil[2]					4	2	62	20	10
High oleic rapeseed oil[2]					4	2	75	14	2
Sunflower oil[2]					6	4	30	59	
High oleic sunflower oil[2]					4	4	80	10	

Table 2.3: Main typical fatty acid composition of vegetable oils, divided in saturated, monounsaturated and poly-unsaturated fatty acids (Product specifications AAK AB, 2012).

Vegetable oil	SFA	MUFA	PUFA
Coconut oil	92	6	2
Palm olein	45	43	11
Rapeseed oil	7	63	30
High oleic rapeseed oil	7	77	16
Shea stearin	64	33	3
Sunflower oil	10	30	59
High oleic sunflower oil	10	80	10

2.3.1 Coconut Oil

The coconut palm, *Cocos nucifera*, grows in the tropical areas of the world and can be up to 25 meters in height, with a crown of large pinnate leaves. The palm can tolerate high levels of salt and grows mainly in coastal regions, where the major parts of the plantations are located. It produces mature fruits in a period of seventy years from six years of age. The fruits as well as the trees are used as foods and drinks, building materials, ropes and mats. Coconut oil is extracted from dried coconut fruit flesh called copra, which contains about 65 % fat and only 5-7 % water. The main cultivation areas and the largest export countries of coconut oil are the Philippines and Indonesia (AAK AB, 2007).

Coconut oil consists of almost exclusively SFAs, with the main fatty acids being lauric and myristic acid (AAK AB, 2007; Arvanitoyannis et al., 2010). The fat is used in the food industry in ice cream, margarine and confectionary fillings, as well as in the oleochemical industry for fabrication of fatty alcohols and soaps (AAK AB, 2007).

2.3.2 Palm Oil

The oil palm, *Elaeis guineensis*, originates from West Africa and is a large pinnate-leaved palm that can become 30 meters high and 200 years old in the wild. At plantations the trees are usually replanted when the height has reached 10 meters, which occurs when the palms are about 25 years of age (Corley & Tinker, 2003). The oil palm produces fruit from the third year until it is sixty years old, with full production from its twelfth year (AAK AB, 2007). The small fruits vary between 2 and 5 cm in length and are spherical to ovoid in shape (Corley & Tinker, 2003). The fruits, that are orange-brown in colour when ripe, grow in infructescences with each bunch containing up to 2000 fruits and weighing 50-60 kg (AAK AB, 2007). The fruit consists of a central hard-shelled nut surrounded by an outer pulp, from which the palm oil is extracted. The fruit flesh of the pulp has an oil content of 40 % that is extracted by pressing and centrifugation within days of harvest. Another type of oil, palm kernel oil, is extracted from the nut containing the palm kernel. The main producing and exporting countries of palm oil are Malaysia and Indonesia, where large plantations for systematic cultivation have developed (AAK AB, 2007; Corley & Tinker, 2003).

Palm oil contains almost equal proportions of saturated and unsaturated fatty acids, with palmitic and oleic acid as the most common ones, making the fat semi-solid at room temperature (AAK AB, 2007; Arvanitoyannis et al. 2010). Palm oil is often used in a wide range of food products and often processed into different fractions to maintain the requested properties. The stearin fraction, with high melting point, is mainly used as solid component in margarine, bakery and confectionary, whereas the lower melting point fraction, olein, primarily is used for cooking and salad oils, and for deep fat frying (AAK AB, 2007; Corley & Tinker, 2003).

2.3.3 Rapeseed Oil

Rapeseed, *Brassica napus*, is a bright yellow-flowering member of the *Brassicaceae*, or mustard, family that grows to a height of 75-175 cm. It originates from India and are today cultivated in large parts of the world for the production of animal feed, vegetable oil for human consumption and biodiesel (AAK AB, 2007; Arvanitoyannis et al., 2010). The small, round seeds are black-red in colour and have an oil content of just above 40 %, which is commonly extracted by pressing. Rapeseed is frequently cultivated in Europe, but also in China, India, Canada and Australia (AAK AB, 2007).

Rapeseed oil is nutritionally well balanced, with a very low content of saturated fatty acids and a high content of the unsaturated fatty acids oleic, linoleic and linolenic acid. The content of sterols and tocopherols are relatively high in rapeseed oil. A variety of rapeseed oil with alternating fatty acid composition has been developed by conventional breeding techniques (AAK AB, 2007). High oleic rapeseed oil is consisting of mainly oleic acid and linoleic acid. Due to the low content of linolenic acid, high oleic rapeseed oil is considered a high stability oil compared to normal rapeseed oil, and it is suitable to use in high temperatures (Product specifications AAK AB, 2012).

2.3.4 Shea Fat

The shea tree, *Butyrospermum parkii*, grows wild in West Africa in the savannah region. It is a drought resistant long-lived tree that can be 25 meters in height and produce nuts up to an age of 200 years, and is most productive between 50 to 100 years of age. The fruits are elliptical to round-shaped and about 4 cm long. When ripening, it changes colour from green to yellow-brown and falls to the ground. The work of collecting and preparing the nuts, as

well as kneading the shea butter, is performed mainly by the women in the native countries where the shea trees grow, and the nut has been of great importance for the people of these parts of Africa for a long time when used in food, as lamp fuel and for soap production. Today, the main cultivation and exporting countries of shea fat are Ghana, Burkina Faso and the Ivory Coast (AAK AB, 2007).

Shea fat is composed mainly of stearic and oleic acid, but also to a small extent of palmitic and linoleic acid. The triglyceride stearic-oleic-stearic, is very common in shea fat and by fractionation these triglycerides can be concentrated to be used as cocoa butter equivalence in chocolate. Shea butter is also used in skin care products, as it contains high amounts of non-glyceride components (AAK AB, 2007).

2.3.5 Sunflower Oil

The sunflower, *Heliantus annuus*, originates from the tropical parts of Central and South America and grows today in parts of the world with warm and sunny summer months. The annual crop has a stem that can grow to a height of 2-4 meters and a yellow inflorescence to a diameter of 15-50 cm. Up to 2000 seeds develop in the middle of each flower, and the shape and colour differ depending on variety. The oil content in the seeds is about 40 % and the oil is extracted by crushing or pressing. The main cultivation and exporting areas of sunflower oil are Argentina, Russia, Southern and Eastern Europe and the US (AAK AB, 2007).

Sunflower oil consists mainly of linoleic acid and also oleic acid, and is commonly used in mayonnaise and salad dressings (AAK AB, 2007). Through conventional breeding techniques, several types of sunflower oil with different fatty acid composition have been developed (AAK AB, 2007; Arvanitoyannis et al. 2010). High oleic sunflower oil consists of 80 % oleic acid and only 10 % linoleic acid, making it suitable in applications with high demands on oxidative stability (AAK AB, 2007).

2.3.6 Lipids and Health

Fat is an important part of our diet that gives us more energy per unit weight than other nutrients (9 kcal/g). Lipids make up a major part in our cell membranes and are necessary for the body to make and repair cells, as well as to produce hormones and other hormone like compounds. Fat enables uptake of the fat-soluble vitamins A, D, E and K and are involved in controlling our blood pressure, blood coagulation and immune system (SLV, 2011).

To enable transport of lipids such as triglycerides and cholesterol in the blood, these molecules are combined with proteins to make spherical, water soluble lipoproteins. The lipoproteins consist of an outer shell of proteins, phospholipids and cholesterol molecules surrounding an inner core of triglycerides and other lipids. They transport lipids in the body and are named according to their size and density. The smallest lipoproteins, Chylomicrons, are formed in the absorptive epithelial cells of the small intestine and transport dietary lipids to adipose tissue for storage. Very low-density lipoproteins (VLDLs) transport triglycerides made in the liver cells to adipose cells for storage. VLDLs are converted to Low-density lipoproteins (LDLs) after some of their triglycerides have been deposited in adipose cells. LDLs carry about 75 % of the total cholesterol in blood and deliver it to cells throughout the body for use in repair of cell membranes and synthesis of steroid hormones and bile salts. Excess cholesterol is removed from body cells by High-density lipoproteins (HDLs) and transported to the liver for elimination (Tortora & Derrickson, 2010).

LDLs deposit cholesterol in and around smooth muscle fibers in arteries when existing in excessive numbers. This forms fatty plaques that increase the risk of coronary artery disease, making the cholesterol in LDLs known as the bad cholesterol. On the contrary, HDL cholesterol is known as the good cholesterol, since it prevents accumulation of cholesterol in the blood. Consequently, high levels of LDLs are associated with increased risk of atherosclerosis and coronary artery diseases, whereas high levels of HDLs are associated with decreased risk (Tortora & Derrickson, 2010).

The composition of the fat we are eating influences the concentration of plasma lipoprotein cholesterol fractions at different ways. The saturated fatty acids lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids increase the levels of LDL, whereas stearic (C18:0) acid has no effect (FAO, 2010). The Food and Agriculture Organization of the United Nations, FAO, has concluded that there is convincing evidence that replacing SFAs (C12:0-C16:0) with unsaturated fatty acids, specifically PUFAs, reduces LDL cholesterol concentration and the total/HDL cholesterol ratio. A high intake of SFAs leads to a possible increased risk of diabetes, while increased intake of PUFAs possibly reduces the risk of diabetes. Replacing SFAs with PUFAs has shown to decreases the risk of coronary heart diseases. Very high consumption of PUFAs may increase lipid oxidation, especially when tocopherol intake is low. However, there is insufficient evidence for establishing any relationship of PUFA consumption with cancer (FAO, 2010).

The Swedish National Food Agency recommends that the total energy intake from fat should be 25-30 %, whereof the consumption of SFAs should be limited and contribute to maximum 10 energy percent (E%). MUFAs should contribute to 10-15 E% and PUFAs to 5-10 E%, of which 1 E% from n-3 fatty acids. Linoleic acid and alpha-linolenic acid are essential fatty acids that cannot be synthesised by humans and therefore 3 E% needs to be consumed daily. Higher intake than 10 E% of PUFA is not recommended, since there are no health benefits with higher consumption, but it could possibly increase the risk of peroxidation of fatty acids (SNR, 2005).

2.3.7 Production and Processing

Vegetable oils are used in a wide variety of food products, putting many requirements to the finished oil in order to guarantee a high quality product. Some of the most important properties are bland taste, long shelf life, bright colour and suitable crystallisation and melting behaviour. This is achieved by multiple refining steps when the oil has been extracted from the plant. Extraction is performed commonly by pressing, a physical separation method applied to seeds with an oil content exceeding 25 %. Seeds containing less fat, such as soybeans, needs addition of a solvent, normally hexane, in order to enable fat separation from the solid matters of the seed (AAK AB, 2007).

The refining processes are performed in order to remove unwanted components from the oil, such as free fatty acids, phospholipids, oxidation products, solid particles, coloured compounds and substances with strong taste and odour (AAK AB, 2007; Corley & Tinker, 2003). Neutralisation, bleaching and deodorisation steps make the refined oil pure and bright, with a bland taste and a good shelf life, while sterols and tocopherols are still intact (AAK AB, 2007).

Additional modification procedures, such as fractionation, can also be used to maintain the functionality of an oil needed for a specific application. During fractionation the oil is separated into different parts called fractions, consisting of different triglycerides with

different melting properties. The triglycerides are separated by partial crystallisation as a result of the proportions of more or less saturated triglycerides. The two most common fractions are stearin, consisting of more saturated triglycerides, and olein with lower melting point and more unsaturated triglycerides (AAK AB, 2007; Coultate, 2009; Corley & Tinker, 2003).

2.3.8 Environmental Aspects

According to a life cycle analysis performed by SIK 2008, the main environmental effects from vegetable oils come from the cultivation stage, while transports and industrial processing have less impact. The analysis compared the effects of palm oil, soybean oil and rapeseed oil, and it was shown that rapeseed oil cultivated in Sweden is a better choice for the climate, compared to palm oil and soybean oil. In calculations of greenhouse gas emissions, rapeseed oil contributes to about 1500 kg CO_2 equivalents per tonne oil, whereas the same amount for palm oil is almost 2500 kg. An earlier life cycle analysis has shown that palm oil is the best alternative, but the greenhouse gas emissions from palm oil may vary up to five times depending on cultivation area and processing steps. The study also showed that the emission of greenhouse gases was considerably less for rapeseed oil cultivated in Sweden compared to rapeseed oil cultivated in Germany. This difference is due to the intense use of fertilisers in Germany (AAK AB, 2010).

The net environmental impact of palm oil depends on where it is planted. It often grows in areas with high biodiversity and large carbon-storage values. Forest loss at conversion to oil palm plantations may cause increased risk of fire, erosion, soil degradation and greenhouse gas emissions (WWF UK, 2011). To prevent these negative consequences of palm oil cultivation, future oil palm expansion has to be made without deforestation, and according to Fitzherbert et al. (2008), there is enough non-forest land suitable for plantation development to avoid further deforestation, but often it is cheaper and easier to clear forests. Many species are not able to survive in the degraded and fragmented forests left at conversion of land to palm oil cultivations. This is true for the orangutans living on the islands of Boneo and Sumatra, where they have undergone a tenfold loss in population size (WWF UK, 2011). Other examples of species extinction are reported by Fitzherbert et al. (2008), and show that a mean of only 15 % of the species recorded in primary forest were found also in palm oil plantations. As mentioned in the introduction, RSPO work to support production of sustainable palm oil, and Santa Maria AB is one of the supporting members of RSPO (RSPO, 2009; Santa Maria AB, 2012).

2.4 Lipid Oxidation

Rancidity is a chemical deterioration of fats and oils leading to unpleasant odours and flavours. This decomposition can occur by two different mechanism, oxidative rancidity or hydrolytic rancidity (Coultate, 2009; Kristott, 2000).

Oxidative rancidity, or lipid oxidation, is the process where unsaturated fatty acids are degraded in the presence of oxygen, and the products decompose to produce unpleasant off-flavours. Lipid oxidation of foods causes loss of shelf life, palatability and nutritional quality (Kristott, 2000; McClements & Decker, 2008; Reische et al., 2008).

Hydrolytic rancidity occurs when fatty acids are cleaved off from a triglyceride in the presence of water. The liberated free fatty acids results in the development of off-flavours

(McClements & Decker, 2008; Kristott, 2000). The hydrolysis resulting in rancidity can be a spontaneous chemical reaction or catalysed by enzymes. If the reaction is caused by lipases it is called lipolytic rancidity. Short carbon chain fatty acids of the length C4:0-C12:0 cause particularly unpleasant off-flavours with low threshold values. One example is the liberation of lauric acid from coconut oil having soapy flavour (Johansson, 1976; Kristott, 2000).

2.4.1 Mechanism of Lipid Oxidation

Lipid oxidation is often called autoxidation to describe the self-catalytic mechanism generating free radicals from fatty acids in the presence of oxygen. The chain reaction of autoxidation involves three steps; initiation, propagation and termination, and is visualised in equations 2.5 to 2.9. Free radicals, which are atoms or molecules with unpaired electrons, are here denoted with (·) (Coultate, 2009; McClements & Decker, 2008).

Initiation

$$X\cdot + RH \rightarrow R\cdot + XH \quad (2.5)$$

or

$$RH \rightarrow R\cdot + H\cdot \quad (2.6)$$

Propagation

$$R\cdot + O_2 \rightarrow ROO\cdot \quad (2.7)$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot \quad (2.8)$$

Termination

$$R\cdot + ROO\cdot \rightarrow \text{Stable, non-radical products} \quad (2.9)$$

In the initiation step, hydrogen (H) is removed from a fatty acid molecule (RH) usually in the presence of an initiator (X·), to form a fatty acid radical called an alkyl radical (R·). The initiator can be external energy from heat, light or irradiation, or metal catalysts. The highly reactive alkyl radical reacts with triplet atmospheric oxygen (O_2) in the propagation step, generating another highly reactive radical, a peroxyl radical (ROO·). This molecule can react with other unsaturated fatty acids (RH), resulting in the formation of a hydroperoxide (ROOH) and a new alkyl radical (R·). By repeating the process of the propagation, a chain reaction is formed, increasing the number of free radicals in the fat. When the concentration of free radicals is high enough, they react with one another to form stable, non-radical products in the termination step (Arvanitoyannis et al., 2009; Coultate, 2009; McClements & Decker, 2008).

The lipid oxidation process of foods begins with a lag phase of slow reaction rate, followed by an exponential increase in oxidation rate. During the lag phase, the rancidity is not yet detected and the deterioration of the quality of the food has not yet begun. When the reaction enters the exponential phase, the deterioration and formation of off-flavours occurs rapidly (McClements & Decker, 2008). The rate of oxidation and the length of the lag phase of fats and oils depend on the degree of fatty acid unsaturation, presence of oxygen, pro-oxidants or antioxidants, and storage conditions like temperature and presence of light (Kristott, 2000; McClements & Decker, 2008).

The fatty acid radicals formed in the autoxidation process increase with increasing fatty acid unsaturation, because the initiation occurs at the carbon that requires least energy for a

hydrogen atom removal. A carbon atom next to an electron-rich double bond has a weaker carbon-hydrogen covalent bond compared to a saturated carbon without a neighbouring double bond. In polyunsaturated fatty acids, the carbon-hydrogen bond of the atom in between two double bonds is even further weakened by withdrawing electrons. With decreased carbon-hydrogen bond dissociation energy, hydrogen abstraction becomes easier and oxidation occurs faster. This explains the increased oxidation rates of oleic, linoleic and linolenic acids, respectively (Kim & Min 2008; McClements & Decker, 2008).

2.4.2 Pro-oxidants

Pro-oxidants are substances or factors that cause or accelerate lipid oxidation, either by direct interaction with unsaturated fatty acids to form lipid hydroperoxides, or by promoting the formation of free radicals. Some pro-oxidants present in food systems are transition metals, singlet oxygen, light and increased temperature (McClements & Decker, 2008).

Transition Metals

Transition metals are metals with two or more valence states, for example iron, copper, manganese, nickel and zinc. They are one of the most important pro-oxidants in foods since they exist in all biological materials and are common constituents of packaging material. They have the ability to abstract a hydrogen atom from fatty acids, thereby forming alkyl radicals and initiating lipid oxidation. Transition metals also have the ability to decompose hydroperoxides into free radicals through a reduction-oxidation reaction. The most common transition metals present in foods causing these reactions are iron and copper. The pro-oxidative ability of metals depend on their redox state, and generally the reduced state is more reactive than the oxidised state (McClements & Decker, 2008).

The content of metal ions, and enzymes and pigments also being identified as lipid oxidation catalysts, can be reduced in fats and oils by bleaching and refining processes. To avoid metal contamination it is also important to avoid processing equipment and packaging materials containing metals (Kristott, 2000).

Singlet Oxygen

Singlet oxygen ($^1O_2$) is a short-lived, highly reactive and highly energetic form of oxygen that can react directly with the double bonds of unsaturated fatty acids in the formation of hydroperoxides, without the formation of free radicals (Coultate, 2009; Kim & Min, 2008). In this way, singlet oxygen can cause and accelerate lipid oxidation (Coultate, 2009; McClements & Decker, 2008). Singlet oxygen is produced from ordinary triplet oxygen ($^3O_2$) by photosensitisation in the presence of light. A photosensitiser present in the food, such as chlorophyll, riboflavin or myoglobin, can absorb energy from light and transfer it to convert triplet oxygen into singlet oxygen (Kim & Min, 2008; McClements & Decker, 2008). The reaction rate of singlet oxygen is related to the number of double bonds in the molecules, and occurs much faster than triplet oxygen lipid oxidation (Kim & Min, 2008).

Light and Elevated Temperature

The presence of light as well as increased temperature can initiate autoxidation and promote decomposition of hydroperoxides to produce free radicals. Therefore it is important to store foods cool and in the absence of light (McClements & Decker, 2008).

2.4.3 Antioxidants

Antioxidants are substances able to delay the initiation or reduce the rate of oxidation reactions (Kristott, 2000; Nawar, 1996; Reische et al., 2008). Antioxidants can be naturally present in foods or intentionally added in order to maintain the quality and extend the shelf life of a food product. Antioxidants can be classified by their mechanism of action in two groups; primary and secondary antioxidants (Reische et al., 2008).

Primary Antioxidants

Primary antioxidants are also called chain-breaking antioxidants and have the most commonly occurring antioxidant mechanism. These antioxidants delay or inhibit the initiation of oxidation by interfering with the propagation phase of autoxidation (equation 2.8) (Reische et al., 2008). The primary antioxidants react with lipid radicals and peroxyl radicals, thereby inhibiting the free radical chain reaction. This is done by abstraction of the unpaired electrons of the fatty acid radical (ROO ·) and by donation of a hydrogen atom from the antioxidant molecule (AH), as described in equation 2.10.

$$ROO\cdot + AH \rightarrow ROOH + A\cdot \quad (2.10)$$

The antioxidant radical produced (A ·) is stabilised by delocalisation of the unpaired electron, thus preventing it from react to form new fatty acid radicals (Kristott, 2000; Nawar, 1996; Reische et al., 2008).

Primary antioxidants are compounds containing one or more phenolic groups, with the molecular structure of the phenolic ring forming stable resonance intermediates, without positions suitable for attack by molecular oxygen (Nawar, 1996; Reische et al., 2008).

The most common primary antioxidants are the tocopherols, containing various methylated phenols, present in various amounts in vegetable fats and oils. Tocopherols are retained in the refining process, but some are lost during the deodorisation, making a careful process important in order to preserve the antioxidants (Kristott, 2000; Reische et al., 2008). Tocopherols are used commercially as a natural antioxidant, being derived from the soybean oil refining industry (Reische et al., 2008).

Secondary Antioxidants

Secondary antioxidants are preventive antioxidants which inactivate oxidation catalysts by a number of different methods, for example as metal chelating agents or as singlet oxygen quenchers (Reische et al., 2008). Chelating agents, such as citric acid, form a complex with a transition metal ion catalyst, thereby decreasing the pro-oxidative effect of the metal by reducing the redox potential and stabilising the oxidised state of the metal (Kristott, 2000; Reische et al., 2008).

Other antioxidants, such as beta-carotene, lycopene and other carotenoids, reduce oxidation by reaction with singlet oxygen ($^1O_2$). The exchange energy of the singlet oxygen is transferred to the carotenoid, returning the highly reactive molecule to its inactivated triplet state ($^3O_2$), while the carotenoid releases the energy as heat (Coultate, 2009; Reische et al., 2008).

Secondary antioxidants can act synergistically to promote the antioxidant activity of primary antioxidants (Reische et al., 2008).

2.4.4 Formation of Lipid Oxidation Decomposition Products

The hydroperoxides formed at autoxidation are generally colourless, odourless and tasteless, not directly contributing to rancidity. However being relatively unstable, particularly at elevated temperatures, these primary oxidation products decompose to form small, volatile compounds causing the off-aromas of oxidative rancidity. These secondary oxidation products contain a wide range of compounds, such as hydrocarbons, aldehydes, ketones, alcohols, esters and acids, due to the many different possible reaction pathways (McClements & Decker, 2008; Min, 2000).

The complex mixture of produced volatile compounds acts individually or in combination to generate unique tastes and aromas, and the same compounds can generate different sensory impressions in different food products (Jacobsen, 2010; Min, 2000).

Each flavour compound has a different flavour threshold value. Unsaturated aldehydes and ketones have the lowest sensory thresholds and are therefore the major contributors to the undesirable and unpleasant odours and flavours in oxidised food products (Min, 2000). The compounds with the highest thresholds are hydrocarbons such as alkanes and alkenes, with threshold values thousand-folds the ones of ketones (Jacobsen, 2010; Min, 2000). This wide range of sensory thresholds makes it important to consider not only the concentration of a volatile compound present, but also the threshold values, when studying the overall flavour of oxidised oil. A compound with a low threshold value can have a significant influence on the flavour perception even in small quantities, in opposite to a compound present in larger quantities but with a higher sensory threshold value (Kim & Min, 2008; Min, 2000).

Lipid oxidation of fats and oils can give rise to several different off-flavours and off-aromas caused by a wide range of volatile oxidation compounds. They can range from cardboard, green, grassy, beany and nutty to tallow, metallic, rancid, synthetic and painty. Other flavours can be oily, fishy, deep-fried, lemon, fruity, soapy and bitter (Jacobsen, 2010; Min, 2000; Reineccius, 2006; Ridgway & Lalljie, 2011).

2.4.5 Sensory Evaluation of Lipid Oxidation Products

The various and complex substances formed as lipid oxidation products can be evaluated and measured in many different ways. The primary oxidation products are colourless and flavourless whereas the secondary decomposition products give rise to both flavours and odours, and are therefore a distinct indication of lipid oxidation and food deterioration. These processes can be assessed by sensory evaluation or by several analytical methods (Shahidi & Wanasundara, 2008).

Sensory evaluation is considered the most appropriate and best suitable method to assess the quality of fats and oils. This is due to that the human senses are very sensitive and are able to detect the off-aromas and off-flavours produced by lipid oxidation directly and at very low concentrations (Gordon, 2001; Kristott, 2000; McClements & Decker, 2008). However, sensory analysis needs to be performed with a trained panel able to detect small changes in rancidity, and the training is usually product specific due to that the wide range of oxidation products generates different sensory profiles depending of the fatty acid composition. This makes sensory analysis a time consuming and often costly technique (Kristott, 2000; McClements & Decker, 2008). The reproducibility of sensory evaluation is not as good as that

of analytical methods and combining these two types of evaluation techniques would give the best results (Gordon, 2001; McClements & Decker, 2008).

Sensory analysis evaluates the attributes appearance, aroma, texture and flavour of a food item and how they are perceived. This technique is usually applied in the fields of quality control, product development and research, to conduct valid and reliable information in order to make good decisions about the perceived sensory properties of the product (Meilgaard et al., 1999).

Sensory analysis can be divided into two parts, consumer tests and analytical tests. The consumer tests intend to determine the consumers' approval of a product and can be either qualitative or quantitative. The qualitative tests include a small number of respondents and consist of focus groups, interviews or questionnaires. The quantitative tests demand a larger number of respondents and are often designed as a nine grade hedonic scale, with the alternatives "like extremely" to "dislike extremely" (Lundgren, 1981; Wendin, 2011). From the received data it is then possible to calculate which product the consumers prefer. In consumer testing it is important that the respondents are representative to a certain group of consumers, and not trained in judging food products. A consumer test can also be designed as a preference test where the preferred of two different samples are to be chosen (Lundgren, 1981).

Analytical tests demand selected and trained panelists able to detect small differences, and can be divided into difference tests and descriptive tests (Lundgren, 1981; Wendin, 2011). With a difference test it is possible to detect if there exist a sensory difference between samples or not. The three most common methods to investigate this are triangle test, pairwise test and duo-trio test. A triangle test contains three samples from which two are identical, and the panelists are to decide which sample that differs from the others. In a pairwise difference test the task is to decide if two different samples differ in a certain sensory attribute, for example which sample that has the saltiest taste. A duo-trio test aims to select which of two samples that is identical to a presented reference sample, in order to decide if there exists a difference between two products (Lundgren, 1981). A descriptive test may conclude which differences that exist between samples, and how significant these differences are. The test is performed by making a list of the products' sensory attributes of interest and to select a scale to be able to measure the intensity of each attribute (Lundgren, 1981). This gives the sensory profile of the product, a description of the sensory properties of a sample assigned with intensity values of each attribute. A profile obtained by agreement after discussion by a group of assessors, after each assessor has evaluated the product by themselves before the discussion, is called a consensus sensory profile (ISO 13299, 2010).

To obtain the best evaluation, the sensory analysis is performed individually with the assessors seated in booths in a quiet, well ventilated room (Lundgren, 1981). However, at a consensus profiling, the assessors are seated around a table. The main advantage with consensus profiling is that it is possible to test many samples at a relatively low cost in both samples and assessors' time. Usually a descriptor panel consists of eight to twelve assessors, but in consensus profiling the number of panelists may be as few as four (ISO 13299, 2010).

The intensity of a perceived attribute can be expressed by numbers or words using a response scale, which can be either continuous or discrete. A continuous scale allows the assessor to register a response anywhere on a straight line scale, whereas a discrete scale is composed by a certain number of predefined steps (ISO 4121, 2003; Lundgren, 1981). The discrete

response scale has to be broad enough to cover the full range of intensities, with enough scaling steps for the assessors to express small differences. However, the steps have to be few enough to avoid that the assessors perceive the task as too difficult (Lundgren, 1981; Meilgaard et al., 1999). Usually the scales have five to nine points which can reach from ”nothing” to ”very strong” when describing the intensity of an attribute. The variety of food products require that a new formulary, with specific attributes and response scale is made for every product evaluation to be performed (Lundgren, 1981).

Sensory evaluation is widely used in the food industry to determine whether product differences result from the effects of processing conditions, storage time, different packaging materials or a change in ingredients (Meilgaard et al., 1999; Min, 2000). When there are several samples to evaluate and to control during a product or process development, it can be suitable to perform a screening test, a preliminary selection procedure, in order to decide which samples are to be included in a complete sensory profiling (ISO 5492, 2009).

2.4.6 Analytical Measurements of Lipid Oxidation Products

There are several different analytical methods to measure lipid oxidation in foods. Because of the complexity of the lipid oxidation decomposition products there is no uniform or standard method to detect the oxidative changes in a food system. Each test method detects a single group of chemical compounds, and due to the wide range of the oxidation products formed, it is important to decide which components to observe. To maintain a characterisation of the degree of oxidation of an oil, at least two chemical tests have to be performed (Gordon, 2001; Kristott, 2000; Shahidi & Wanasundara, 2008). The most commonly used chemical tests to evaluate the quality of oils are peroxide value, *para*-anisidine value, thiobarbituric acid value and chromatographic methods (Kristott, 2000).

Peroxide Value

The content of primary products of lipid oxidation, hydroperoxides, can be measured by the peroxide value (PV). The PV is determined by measuring the amount of iodine formed when the hydroperoxides liberate iodine ions from potassium iodide. The amount of iodine is proportional to the concentration of hydroperoxides in the sample. PV is expressed in milliequivallents of oxygen per kilogram of fat or oil, meq/kg, and the method is appropriate to use in the beginning of the oxidation process, since the content of hydroperoxides will increase to reach a peak and then decline when they decompose to secondary oxidation products (Nawar, 1996; Shahidi & Wanasundara, 2008). For this reason the PV may be low even though the oil is very rancid, and a PV measurement should therefore be combined with a technique detecting secondary oxidation products to maintain a more correct representation of the oxidation process (Gordon, 2001; Kristott, 2000).

para-Anisidine Value

The *para*-anisidine value (AV) is a measurement of the content of aldehydes in fats and oils, formed as secondary products in lipid oxidation. *para*-Anisidine is a reagent that reacts with aldehydes in the presence of acetic acid to form yellowish products with an absorption maximum of 350 nm (Gordon, 2001; Nawar, 1996; Shahidi & Wanasundara, 2008). The AV is defined as the absorbance of a solution containing 1 gram oil in 100 ml of a mixture of the solvent isooctane with *para*-anisidine in 0.25 % acetic acid (Gordon, 2001).

Thiobarbituric Acid Value

A common method to evaluate lipid oxidation in foods is the thiobarbituric acid (TBA) test. One of the secondary products of lipid oxidation is malonaldehyde, and this molecule can react with TBA to form a pink-coloured complex with an absorption maximum of 530 nm. This reaction is however not specific and other aldehydes may also react with TBA, forming complexes with other absorption maximum. To emphasise the variety of products detected in this test, the TBA value is often expressed as TBA reactive substances (TBARS) or as malonaldehyde equivalents per kilogram sample (Gordon, 2001; Nawar, 1996; Shahidi & Wanasundara, 2008).

Chromatographic Methods

Several different chromatographic techniques, including high-performance liquid chromatography (HPLC) and gas chromatography (GC) can be used to detect oxidation products in fats, oils and foods. These methods are based on separation and measurements of relative quantities of different fractions, such as volatile compounds or individual substances (Nawar, 1996). Coupling the chromatograph with mass spectrometry (MS) enables direct characterisation of both primary and secondary oxidation products, with high sensitivity (Shahidi & Wanasundara 2008). It has been shown that it is possible to compare sensory evaluations and analysis using GC with high correlation (Min, 2000). These kinds of methods requiring high-technological equipment are however not suited for routine quality assessment of oils but for research purposes (Kristott, 2000).

3 Methods

The following sections contain the methodology used in this project. The chapter is divided in two parts; Vegetable Oils and Spice Mixtures, each section being subdivided to enhance the understanding of the arrangements of the different methods used.

3.1 Vegetable Oils

Nine different vegetable oils were chosen to be investigated as possible replacement to palm oil as processing aid in the manufacturing of spice mixtures. Palm oil was also included in all experiments as a reference. The ten oils included in this Master's Thesis are presented in table 3.1.

Table 3.1: The ten vegetable oils included in the accelerated stability tests.

Vegetable oils included in the stability tests
Palm oil
Rapeseed oil
Rapeseed oil + antioxidant
High oleic rapeseed oil
High oleic rapeseed oil + antioxidant
Sunflower oil
High oleic sunflower oil
Rapeseed + sunflower oil
Coconut oil
Shea stearin

The examined rapeseed oil is a Swedish product with good nutritional composition, as described in section 2.3.3. The disadvantage with rapeseed oil is that it is not very stable due to a high content of polyunsaturated fatty acids, therefore a more stable variety of rapeseed oil, high oleic rapeseed oil was included in the tests. In order to investigate whether these oils would be better preserved with the addition of an antioxidant, they were also tested with the addition of a mixture of tocopherols and gallic acid. Gallic acid origins from leaves, fruits and galls of several different plants (Reische et al., 2008).

Sunflower oil and high oleic sunflower oil were also part of the test, since sunflower oil is nutritionally well balanced and has shown to be somewhat more stable compared to rapeseed oil (Bringsarve, 2012). A mixture of rapeseed oil and sunflower oil is used in the manufacturing of tortilla chips at Santa Maria AB, and this oil was also part of the test. Coconut oil is a stable oil and in similarity with palm oil it is semi-solid at room temperature. Shea stearin, a solid fraction of shea butter, was included with the intention to be mixed with high oleic rapeseed oil to maintain a mixture with higher melting point. Due to that this combination would require an extra mixing step performed in the spice factory, this alternative was early being rejected. However, shea stearin was a part of the stability test throughout the testing period.

Samples of each oil were obtained from AarhusKarlshamn AB (AAK AB).

3.1.1 Accelerated Shelf Life Test

To evaluate the stability of the ten different oils, ASLTs were performed. 25 samples of each oil were prepared by measuring 30 grams of oil in 81 ml glass jars, used as retail packaging for spices (figure 3.1). Six samples of each oil were placed in three different heat chambers, at 60°C, 40°C and 31°C, respectively. The same number of samples were also stored at room temperature, approximately 22°C. One sample of each oil was collected from the cabinets every second week in twelve weeks, to be evaluated by sensory analysis. Malcolmson et al. (1994) point out the value of having the same headspace volume for all samples, because the ratio of surface exposed to oxygen to oil volume affects oxygen accessibility. This aspect was considered when all samples contained the same amount of oil.

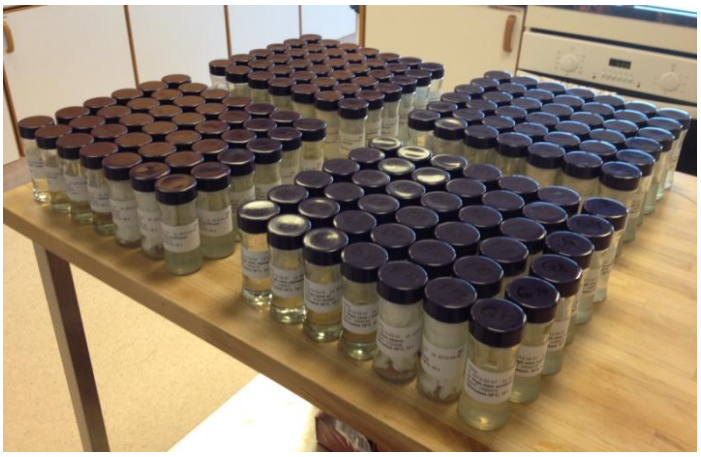

Figure 3.1: Some of the oil samples packed in glass jars included in the storage stability test.

The temperatures of the ASLT were decided after the following considerations. Broadbent and Pike (2003), Malcolmson et al. (1994) and Robertson (2008) all used the temperature 60°C to deteriorate rapeseed and other vegetable oils in accelerated tests, therefore this temperature was chosen as the highest temperature. 40°C and 31°C were used since these are the temperatures normally used by Santa Maria AB when conducting shelf life tests of products. Samples stored in room temperature, 22°C, were used as references. Taoukis and Labuza (1996) highlight the importance of keeping the relative humidity constant at shelf life testing when placing food samples in environmental cabinets at different temperatures, due to the effect of the water activity. During these experiments, the relative humidity was kept identical in the three heat chambers since they were all placed in the same room. However, the relative humidity varied naturally over time.

3.1.2 Sensory Analysis

In order to evaluate the stability of each vegetable oil the samples were analysed by sensory evaluation throughout the ASLT. Prior the accelerated storage, the appearance, aroma, flavour and texture of all oils were evaluated, to maintain a sensory profile of each oil.

The sensory evaluation was performed by an internal panel at Santa Maria AB consisting of mostly four assessors. Due to the numerous amounts of samples to be analysed, the sensory evaluation was performed as a screening test evaluating the attributes of rancidity of each sample, and not as a complete sensory profiling. The scale used was a discrete scale consisting of five steps, described both by numbers and a descriptive text, as presented in table 3.2.

Table 3.2: The discrete intensity scale used in the sensory analysis.

1	**None**	When you have the sample in your mouth you cannot perceive the attribute at all
2	**Weak**	You have to try hard to perceive and find the attribute, and when it is there the intensity is weak
3	**Distinct**	You perceive the attribute immediately when you have the sample in your mouth and the intensity is distinct
4	**Strong**	You perceive the attribute immediately and the intensity is strong
5	**Very strong**	You perceive the attribute immediately and the intensity is very strong

Before starting the evaluation, the assessor panel was trained to analyse other oil samples of different intensity to declare which intensity corresponding to each step of the scale. This was made for the properties of appearance, aroma and flavour, using different commercial oils, including crude linseed oil and palm oil taken from the factory accelerated in 60°C for three days. The sensory attributes obtained by evaluating these oils were listed and used as a basis when performing the sensory evaluation of each oil sample. Attributes of aroma and flavour were added with time when the character of the oils changed. The attributes obtained throughout the sensory assessment are listed in table 3.3.

About one hour prior evaluation the samples of liquid oils were put in room temperature, whereas shea stearin, coconut oil, and palm oil being solid at room temperature were put in heat chamber. Samples of shea stearin were put in 60°C about one hour to become liquid, whereas samples of coconut oil and palm oil were put in 40°C. About fifteen minutes prior evaluation all samples were put in a water bath of 37°C and this temperature was kept constant throughout the test session. Sometimes it was not possible to evaluate the samples in connection to the collection from the heat chambers. At these occasions the samples were put in refrigerator (4°C) until evaluation.

The assessment was performed in a well-ventilated room intended for taste testing at Santa Maria AB, around a table. The attributes and the intensity of each sample were obtained after discussion in the group of assessors, after each assessor had evaluated the product by themselves before the discussion. To obtain results independent of each other, it is important to get rid of the taste of a sample before testing the next (Lundgren, 1981). This was achieved by drinking warmish water or sparkling water, and eating wheat wafers between each sample.

Table 3.3: The attributes perceived during the sensory analysis, with explanation to intensity.

Appearance	
Yellow	Range from light, almost transparent to very strong yellow colour
Opaque	Range from transparent and clear via cloudy to opaque and white
Aroma	
Cardboard	Range from no aroma to very strong aroma of cardboard
Cereals	Range from no aroma to very strong aroma of cereals
Citrus	Range from no aroma to very strong aroma of citrus
Linseed oil	Range from no aroma to very strong aroma of linseed oil
Maize	Range from no aroma to very strong aroma of maize
Nutty	Range from no aroma to very strong nutty aroma
Stearin	Range from no aroma to very strong aroma of stearin
Flavour	
Bitter	Range from no flavour to very strong bitter flavour
Cardboard	Range from no flavour to very strong flavour of cardboard
Cereals	Range from no flavour to very strong flavour of cereals
Citrus	Range from no flavour to very strong flavour of citrus
Linseed oil	Range from no flavour to very strong flavour of linseed oil
Maize	Range from no flavour to very strong flavour of maize
Nutty	Range from no flavour to very strong nutty flavour
Stearin	Range from no flavour to very strong flavour of stearin

The results of the attributes from the consensus sensory profiling of each oil were translated to intensity of rancidity. Since studies have indicated that odour and flavour intensity scores of sensory analysis are equally effective in depicting differences in rapeseed oil stability (Malcolmson et al., 1994), the results of each oil were plotted as an average of the attributes of both aroma and flavour versus time. Further, an Arrhenius plot was made for each oil to maintain the activation energy as a measure of the temperature dependence of the oil.

3.1.3 Measurement of Peroxide Value and *para*-Anisidine Value

To compare the results of the extent of deterioration of vegetable oils performed by sensory analysis with analytical measurements, samples of rapeseed oil and palm oil were analysed by PV and AV. Samples of rapeseed oil and palm oil kept in refrigerator, and stored for two, four, six and eight weeks at room temperature and 40°C, respectively, were sent to Aarhus Karlshamn AB for analysis of PV using the AOCS Offical Method Cd 8b-90. The samples kept in refrigerator and those stored eight weeks were also analysed by AV using the methods IUPAC Method 2.504 and AOCS Official Method Cd 18-90.

3.2 Spice Mixtures

The following section includes presentation of the selected spice mixtures and the performed methods for analysis.

3.2.1 Selected Spice Mixtures

Four different spice mixtures were chosen for the experiments in this project in order to investigate if addition of different vegetable oils as processing aid had an influence on separation, dusting and caking. The four spice mixtures were Barbeque Honey Seasoning, Fajita Spice Mix, Italian Salad Seasoning and Taco Spice Mix. Each of these products was chosen on different premises and because they have had shown different types of problems, such as dusting and caking. All these four spice mixtures have a shelf life of three years and should be stored in a cool and dry place. Below follows a description of the spice mixtures, with ingredient declarations and sensory profiles described by internal product datasheets.

Barbeque Honey Seasoning

This is a barbeque seasoning with a rich flavour of honey, suitable to add to pork, chicken or salmon at the end of barbequing. This product was chosen due to the relatively high content of oil as a processing aid. The ingredients are salt, paprika, onion, sugar, garlic, tomato, yeast extract, other spices, cayenne pepper, honey flavouring, chili pepper, citric acid, anti-caking agent (E551), maltodextrin and smoke flavouring.

Sensory profile:

Appearance:	Orange powder with red and black pieces.
Aroma:	Star anise, onion and weak honey.
Flavour:	Honey, chili, onion, star anise, salty and sweet with chili heat.
Texture:	Flocculated spice mix of salt and larger particles.

Fajita Spice Mix

This is a seasoning mix for tortilla filling intended to be used with chicken to make a fajita. It was selected due to earlier problems of caking. The ingredients are chili pepper, salt, onion, sugar, cumin, garlic, oregano, lime extract, lemon powder, anti-caking agent (E551), cinnamon, nutmeg, coriander leaf and flavour.

Sensory profile:

Appearance:	Red powder with herb flakes and filaments.
Aroma:	Citrus, smoky and herbaceous.
Flavour:	Salt, citrus and herbaceous with medium heat.
Texture:	Free-flowing, granular powder.

Italian Salad Seasoning

This salad seasoning has a flavour of Italian herbs and garlic and is suitable in salad dressings. It was chosen as a test blend due to its tendency of caking and dusting. The ingredients are salt, onion, dextrose, garlic, leek, other herbs, parsley, oregano and anti-caking agent (E551).

Sensory profile:

Appearance:	Light green powder of herbs and salt.
Aroma:	Herbaceous, aromatic and salt.
Flavour:	Salt, onion, herbaceous, tarragon, sweet.
Texture:	Fine powder of grinded herbs. Salt and herbs are slightly separated.

Taco Spice Mix

This spice mixture is a part of the Santa Maria Tex Mex concept, and is an important product for the company. It is a product to be mixed with minced meat to make tortilla and taco fillings. It was selected because it is an important and high selling product. Taco Spice Mix contains dextrose, salt, onion, modified starch (potato), chili pepper, cumin, garlic, potato fiber, oregano, paprika extract, flavour, anti-caking agent (E551), citric acid, maltodextrin and yeast extract.

Sensory profile:

Appearance:	Light orange-brown powder with slightly larger light orange flecks.
Aroma:	Distinct cumin, chili powder and paprika.
Flavour:	Very sweet, a bit sour taste of cumin, mild chili and garlic. Moderate saltiness.
Texture:	Homogenous, free-flowing powder.

3.2.2 Preparation of Spice Mixtures

In order to evaluate if different oils have an effect on caking, dusting and separation of the four spice mixtures, the seasonings were mixed with three different oils. Rapeseed oil and sunflower oil were chosen mainly due to the convenience of handling and low costs, and palm oil was used as a reference. During the work with this Master's Thesis it was found that the spice plant of Santa Maria AB in Saue, Estonia, use rapeseed oil as processing aid in the spice mixtures produced there (Tammel, 2012). This knowledge also made it interesting to include rapeseed oil as a part of the oils tested in spice mixtures.

Three kilogrammes of each of the four seasonings were manually mixed to ensure that all ingredients were treated in the same way. Each spice blend was divided into four parts, and palm oil, rapeseed oil and sunflower oil was added to three of them, respectively. No oil was added to the last part, to maintain samples functioning as references, to be able to separate differences caused by the different oils and other factors such as the surrounding temperature and relative humidity.

To make the accelerated storage trials as realistic as possible, the mixed spice blends were packed in glass jars or in laminate bags. Barbeque Honey Seasoning and Italian Salad Seasoning are commercially packed and retailed in glass jars, whereas Fajita Spice Mix and Taco Spice Mix are sold in laminate bags. Hence these packaging materials were chosen for each spice mixture, respectively. 40 grams of each seasoning was weighed and packed. The Fajita Spice Mix and Taco Spice Mix were packed in laminate bags having a protective barrier consisting of PE/EVOH/PE, sealed with a manual weld. The Barbeque Honey Seasoning and Italian Salad Seasoning were packed in 81 ml glass jars sealed with a sprinkler cap and a plastic lid.

To investigate the majority of the tested oils, they were mixed with a test mixture consisting only of hygroscopic ingredients. 19.8 % each of the ingredients salt, sugar, onion powder,

tomato powder and yeast extract were mixed with 1 % oil. The test mixtures were packed in laminate bags.

3.2.3 Accelerated Shelf Life Test

To accelerate the shelf life of the spice mixtures containing different oils, ASLT was used. The four spice mixtures were put in heating chambers of 40°C and 60°C and room temperature (22°C) as reference. The same temperatures as during the ASLT for the vegetable oils were used, with the exception of 31°C, to reduce the number of samples and because this temperature did not show any noteworthy acceleration of the oil samples. All samples, including the ones packed in laminate bags, were stored standing to avoid pressure from the weight of other bags if stored on top of each other.

All samples were evaluated after four and eight weeks in all temperatures, with the exception of Taco Spice Mix being evaluated after two weeks instead of four at storage in 60°C. This was due to that the seasoning started caking after only a few days in the high temperature, and therefore was evaluated earlier. Barbeque Honey Seasoning and Italian Salad Seasoning were additionally evaluated after two and six weeks at storage in 40°C. The reason for this was that these spice mixtures was shown to be relatively agglomerated at the first evaluation, and it was decided to perform two additional sample series.

The test mixture consisting of hygroscopic ingredients was stored in room temperature (22°C) and at 40°C. The samples were evaluated after four and eight weeks.

3.2.4 Analysis of Angle of Repose

The method used to evaluate clump formation and flowability was angle of repose measured by a fixed height cone. There are no general descriptions on how to perform the measures of angle of repose. Geldart et al. (2006) have developed a piece of equipment in which the powder is made to flow by a vibrator, letting the powder pour through a funnel forming a semi-cone, whose height and average radius are easy to measure and from which the angle of repose may be calculated or read from a table. The experimental setup used in this project, shown in figure 3.1, is based on pouring the powder into a funnel held at a fixed height above a flat base.

Figure 3.1: Experimental setup to enable measurement of angle of repose of spice piles.

The vibrations necessary to make a cohesive powder pour through the funnel were obtained by gently tapping on the top of the edges of the funnel. The pouring height used in this Master's Thesis was 15 cm, decided from the experiment of Ghazavi et al. (2008), measuring the angle of repose of sand piles using three different pouring heights of 15, 25 and 35 cm. The funnel used for the angle of repose analysis had a height of 7 cm. The diameter at the top was 7 cm and the diameter at the bottom was 1.5 cm. The sample of 40 grams was poured in the funnel while the end was blocked. The sample was poured through the funnel onto a scoreboard, enabling read of radius. If the cohesiveness of the powder made tapping necessary, the number of taps was counted. Each combination of spice mix, oil and storage conditions were present twice, hence there were double samples. Each sample was poured four times through the funnel, producing four different spice piles of one sample. Each pile was photographed from above to enable estimation of the radius and twice from the side, as shown in figure 3.2. Between each photograph, the pile was turned 90° in order to enable estimation of four different angles equally distributed around the pile. The angle of repose was measured using a protractor from the photographs taken, visualised in figure 3.3. If the pile was not symmetrically formed, the initial angle of repose was measured. The grid was used to read the height of each pile.

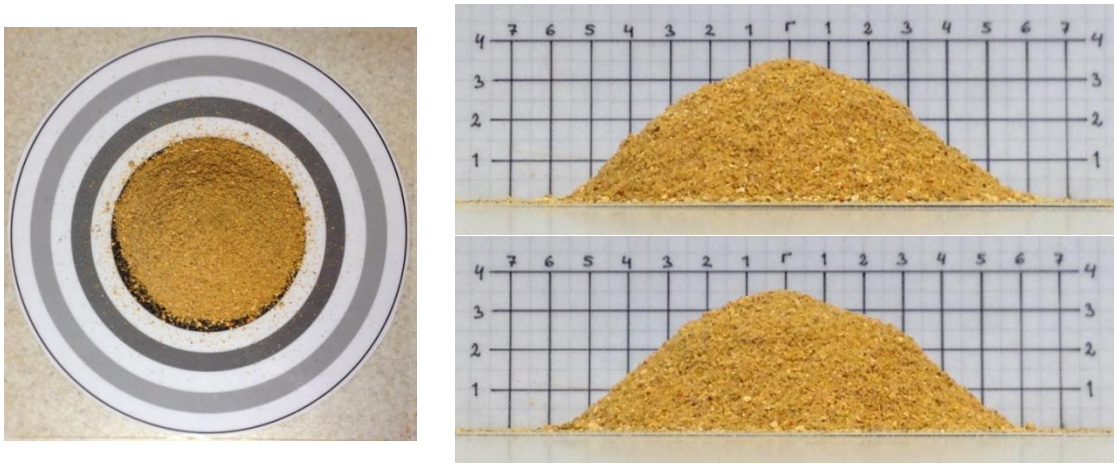

Figure 3.2: Examples of a spice pile photographed from above (left) and from two sides with pile being turned 90° in between (right).

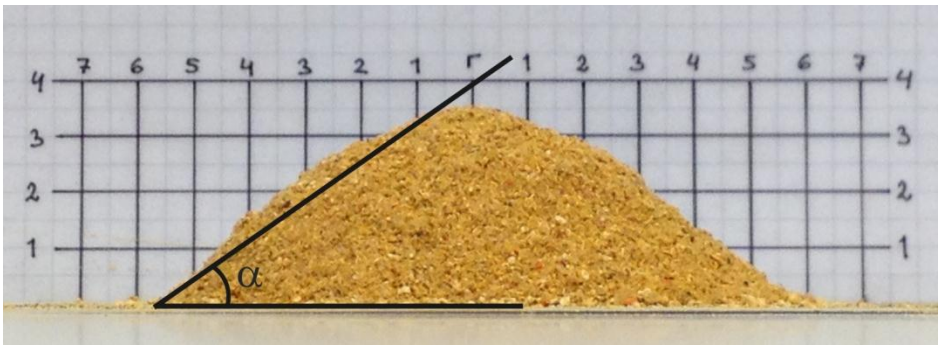

Figure 3.3: Visualisation of the angle of repose measurements. α denotes the angle of repose.

The seasonings were poured directly through the funnel the first two times, without processing, and after the second pile the blend was mixed manually to remove possible clumps, and then poured twice to make two additional piles. Hence, each sample was evaluated by 32 measurements of the angle of repose. The samples of test mixture were treated the same way but were only represented by 16 measurements due to single samples.

3.2.5 Further Evaluation Methods

In order to evaluate separation, dusting and caking of the spice mixtures produced with different oils, they were also analysed visually. The extent of caking and dusting was evaluated on a five grade scale after pouring the samples onto a plate and after manual mixing.

Other ways to evaluate the extent of clump formation or caking was conducted. The samples stored in laminate bags, Fajita Spice Mix and Taco Spice Mix, were weighed before and after storage to investigate whether the seasoning had increased or decreased in weight, to which extent and if this was correlated to the type of vegetable oil. Prior analysis of angle of repose, each bag was cut open at the top and held upside down and the content was allowed to pour out by itself. The amount left in the bag was weighed and once again the bag was held upside down, this time to make sure all content was poured out. The intention of this procedure was

to find a correlation between caking and the amount of spice mixture remaining in the bag after turning it upside down.

As mentioned previously, the spice plant of Santa Maria AB in Saue, Estonia, use rapeseed oil as processing aid in the production of spice mixtures (Tammel, 2012). In order to investigate if it was possible to detect any rancid flavour of products manufactured with rapeseed oil, samples were sent from Saue. Fresh samples were compared with samples long passed the best-before date. This was performed by sensory evaluation both by the pure spice mixtures and by seasoning mixed with water at a concentration of 3 %.

To evaluate if it was possible to identify any rancid taste in foods cooked from spice mixtures containing deteriorated oil, freshly prepared samples of spice mixture was compared to seasoning blended with rapeseed oil stored twelve weeks at 60°C. This was made with minced meat cooked with Taco Spice Mix according to the directions on the packaging, and with chicken fried with 3 % Barbeque Honey Seasoning.

3.3.6 Metal Ion Content

The presence of metal ions, especially iron and copper, may cause lipid oxidation. If the extent of transition metals in the spice mixtures is large, it is possible that components in the seasoning itself induce rancidity. In order to investigate the content of metal ions in the spice mixtures, they were analysed by ion chromatography coupled with UV-vis detection. The analysis was performed according to the method described by Fredrikson et al. (2002). The method is based on that a complexing agent, pyridine-2,6-dicarboxylic acid (PDCA), in the mobile phase forms complexes with the metal ions, which are separated by the chromatographic column. The complexes are postcolumn derivatised with the reagent 4-(2-pyridylazo)resorcinol (PAR), forming complexes detectable by UV-vis absorbance at 500 nm. Prior analysis, 0.2 g of dry sample was digested in 3 ml of H_2O, 0.75 ml of concentrated HNO_3 and 0.5 ml of concentrated HCl in a Teflon vial. The sample was digested in a microwave oven to a transparent solution after the temperature reached 180°C in 15 minutes, and was kept constant for 20 min. Thereafter, the solution was cooled to room temperature and diluted to a final volume of 10 ml in a test tube. Before injection, 0.9 ml of the sample was mixed with 0.1 ml of ascorbic acid to reduce the iron ions from their trivalent to divalent state, making the dilution factor equal to 0.9. 50 µl of sample was injected in the ion chromatograph to be analysed. Figure 3.4 shows the chromatogram of injection of standard solution.

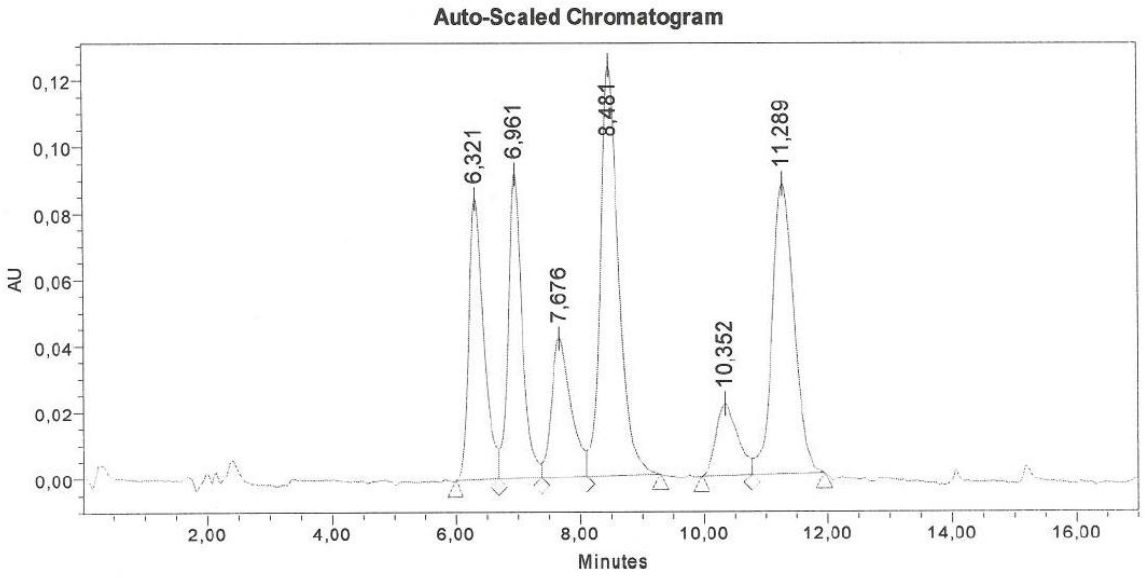

Figure 3.4: Ion chromatogram of the standard solution showing the peaks corresponding to copper, nickel, zinc, cobalt, manganese and iron at different and increasing retention times, respectively.

The metal ion concentration is obtained from the area below the curve, and calculated according to equation 3.1.

$$\frac{Sample\ area\ \cdot\ Standard\ concentration\ (\mu g/ml)\ \cdot\ Volume\ \ (ml)}{Standard\ area \cdot\ Sample\ weight\ (g) \cdot\ Dilution\ factor\ \cdot Dry\ weight\ (\%)} = Ion\ conc.\ (\mu g/g) \qquad (3.1)$$

3.3.7 Test Run in Spice Plant

The experiments and evaluations of this Master's Thesis resulted in the resolution that the best alternative for palm oil used as processing aid would be rapeseed oil. To confirm this, two full-scale test runs were performed in the spice plant. Two batches each of Taco Spice Mix and Italian Salad Seasoning were produced, with rapeseed oil instead of palm oil as processing aid. All ingredients were added as usual, with the only difference of rapeseed oil being manually added instead of automatic addition of palm oil. After mixing for ten minutes, the spice mixtures were packed in large sacks and stored upon retail packaging. Taco Spice Mix and Italian Salad Seasoning were packed at consumer laminate bags and glass jars, respectively. The test run made it possible to evaluate dusting and agglomeration in real production scale.

4 Results and Discussion

This chapter contains the results obtained during this Master's Thesis, as well as a discussion of the outcomes. The chapter is mainly divided in the sections Vegetable Oils and Spice Mixtures, with the result of each experiment presented separately. The first section contains the most relevant and interesting results of each oil presented one by one, and at the end of the section a more comparative and thorough discussion is given.

4.1 Vegetable Oils

To maintain a sensory profile of all vegetable oils included in the stability test, a sensory evaluation was performed on fresh samples at liquid state of a testing temperature of 37°C. The sensory profiles are listed in table 4.1, but due to that the texture of the oil is not relevant for this project, this part of the sensory profile is excluded from the profile.

Table 4.1: Sensory profile of all vegetable oils included in the stability test, performed on fresh samples before storage, at liquid state.

Sample	Appearance	Aroma	Flavour
Palm oil	Transparent, yellow	None	None
Rapeseed oil	Transparent, slightly yellow	None	None
Rapeseed oil + antioxidant	Transparent, slightly yellow	None	None
High oleic rapeseed oil	Transparent, slightly yellow	None	None
High oleic rapeseed oil + antioxidant	Transparent, slightly yellow	None	None
Sunflower oil	Transparent, slightly yellow	None	None
High oleic sunflower oil	Slightly opaque, slightly yellow	None	None
Rapeseed + Sunflower oil	Slightly opaque, slightly yellow	None	None
Coconut oil	Transparent	None	None
Shea stearin	Transparent	Weak cardboard or stearin	Weak cardboard or stearin

Almost all oils are odourless and flavourless, hence they are bland. The exception is shea stearin, having a weak aroma and flavour of cardboard or stearin. The appearance of most oils at liquid state are the same, being transparent and with a weak yellow colour. The exception is palm oil, being more yellow due to relatively high amounts of carotenoids, and coconut oil and shea stearin, lacking yellow colour. High oleic sunflower oil and the mixture of rapeseed and sunflower oils are slightly opaque instead of transparent. This is due to that the waxes naturally present in the oils are not removed during the refining processes (Bringsarve, 2012).

4.1.1 Accelerated Shelf Life Test and Sensory Analysis

The samples stored at different temperatures during the stability tests were evaluated by sensory analysis. In this Master's Thesis, it was found that sensory evaluation is a good but difficult method to use when evaluating deteriorated samples of different vegetable oils. Particularly it has shown to be very difficult to define attributes of flavour and aroma at low intensities. Many foods can be perceived as being odourless, despite the fact that almost all foods and beverages, including fresh water, have an aroma, although the intensity is low and may be difficult to name or categorise. In this sensory evaluation, the aroma of water would not reach to the detectable level of the five grade scale. It turned out that the intensity of rancidity is more important than the attribute of deterioration, and it has shown to be easier to decide the intensity of rancidity than the attribute of the flavour or aroma causing it. The most difficult part of performing sensory evaluation is to agree of an attribute. Assessors associating to different things when perceiving an aroma or a flavour may have difficulties to agree on how to name the attribute, especially if the panel is not trained enough. Individuals may also have different ability to perceive various attributes and also how to express the perception of a certain flavour or aroma. In order to become more coordinated and associate to the same things, the panel should have been more trained and should have tasted a larger amount of samples prior to the actual evaluation. Another way to improve the accuracy of the evaluation could be to provide each assessor with their own sample to analyse, due to that aromas are quickly spread when opening the jar containing the sample. However, this would not be possible when analysing numerous samples.

All oils included in the stability tests were stable enough to not perceive any change in attributes during the twelve weeks assessment at room temperature. For some oils, the attributes perceived during storage at 31°C had low intensities, which did not reach to the second step of perception on the five grade scale. Palm oil and shea stearin had slight basic aromas and flavours during storage at 31°C, but the intensity of each attribute was constant throughout the assessment, making the slope zero. The same was true for the attribute of shea stearin at room temperature. To enable a comparison of the deterioration of all ten vegetable oils, the changes in attributes perceived during storage at 40°C and 60°C are included in the following figures. The oils were found to deteriorate somewhat different compared to each other, generally with formed aromas and flavours of the attributes linseed oil, cardboard and stearin. To maintain an easy comparison of the intensity of deterioration perceived at storage in 40°C and 60°C, the attributes were translated as an average of the intensity of the attributes of both aroma and flavour, to intensity of rancidity.

The deterioration of each oil and the formation of rancid perception were assumed to follow zero order kinetics for all vegetable oils included in the stability test. This was obtained by plotting the intensity of rancidity versus time. With only two temperatures, the determination of the reaction order was not obvious. However, regression analysis showed that zero order kinetics was found to be the best alternative, in spite of the correlation coefficient R^2 ranging from 0.1221, for the mixture of rapeseed and sunflower oil stored at 40°C, to 0.9508 for coconut oil stored at 60°C. The trend line of the rancidity for each temperature was obtained by forcing the intercept through the origin and when the rancidity had a varied intensity with time, only the initial values were included to obtain an appropriate trend line.

The Arrhenius plot, obtained by plotting the natural logarithm of the reaction rate constant, $\ln k$, versus the inverse temperature, $1/T$, gives an indication of the stability of the oil for a change in temperature by providing the activation energy, E_A, from the slope $-E_A/R$. The higher the activation energy, the more the oil is affected by a change in temperature.

The results from the accelerated shelf life tests and sensory evaluations performed to evaluate the samples are presented below, separately for each oil. First, the appearance of the oil at different temperatures during the ASLT is described, and if it was changed throughout the session. The intensity of rancidity, with a discussion of which attributes being perceived as rancid for each oil, is followed by visualisation of the Arrhenius plot. These results give an indication of the temperature stability of the oil.

Palm oil

Palm oil is semi-solid at room temperature, hence completely opaque. The colour is slightly yellow. At higher temperatures; 31°C, 40°C and 60°C, the oil is liquid and strongly yellow without being opaque, as visualised in figure 4.1.

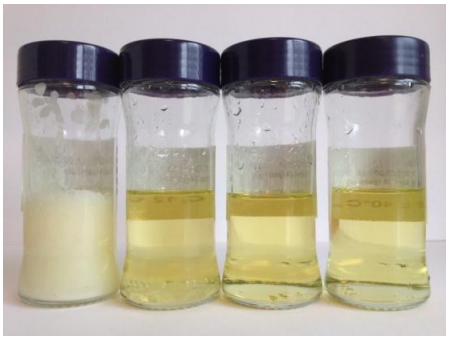

Figure 4.1: Palm oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively. Notice the appearance of the semi-solid sample stored at room temperature.

Palm oil has a bland aroma and flavour at room temperature. During storage at 31°C, it has a slight aroma and flavour of cardboard. At 40°C the perception of cardboard is somewhat more intense and the character changes to linseed oil after twelve weeks. At 60°C the rancidity has the attribute of linseed oil, with the flavour also becoming fairly nutty at ten and twelve weeks. The intensity of these attributes, translated to intensity of rancidity, plotted versus time, and the equation of the trend line of the rancidity of each temperature, are shown in figure 4.2.

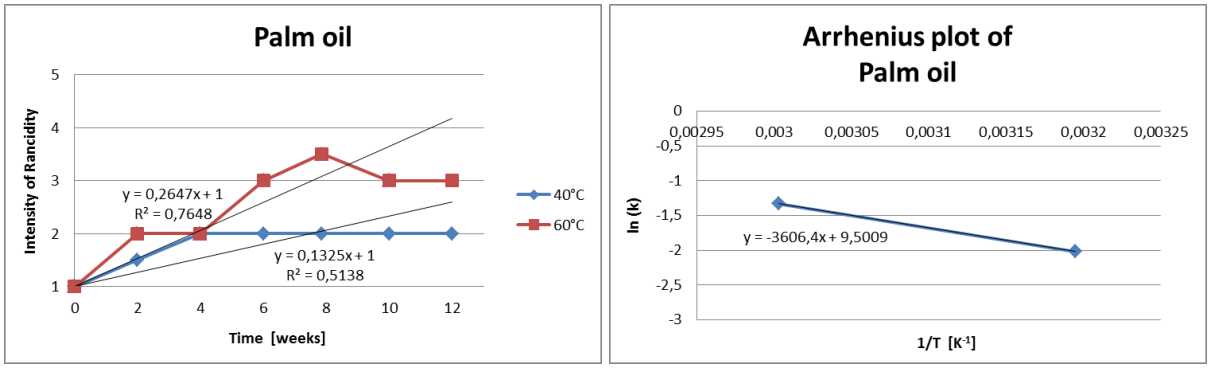

Figure 4.2: The intensity of rancidity of palm oil plotted versus time. The equation of the trend line of each data set can be seen in the figure, as well as the correlation coefficient R^2 (left). Arrhenius plot of palm oil (right).

Due to the fact that only two temperatures could be used to compose the Arrhenius plot, the correlation of the data points is perfect. This does not demonstrate a good correlation with the reality, but in contrast that an increased number of data points are necessary to obtain accurate results. The Arrhenius plot in figure 4.2 enables calculation of the activation energy, E_A, of palm oil to 30 kJ/mol. Extrapolation of the slope of the graph to $1/295$ K^{-1} gives the natural logarithm of the reaction rate constant of the rancidity reaction in room temperature. Exponentiation of this term yields an approximation of the reaction rate constant at room temperature to 0.0656 kJ/mol K. This reaction constant is about 4.0 times smaller compared to the reaction constant of 60°C (0.2647 kJ/mol K), and 2.0 times less than the reaction rate constant of 40°C (0.1325 kJ/mol K) (figure 4.2). This indicates that to reach the same level of rancidity at room temperature as during storage at 60°C and 40°C, it would take four times longer compared to 60°C and twice the time compared to storage at 40°C. Hence, perceiving the rancidity of the same intensity at room temperature as in 60°C and 40°C in twelve weeks, would take twelve months and six months, respectively.

Rapeseed oil

Rapeseed oil is liquid and transparent in all temperatures. The colour is slightly yellow, and becomes somewhat more yellow with time in 60°C, as can be seen in figure 4.3.

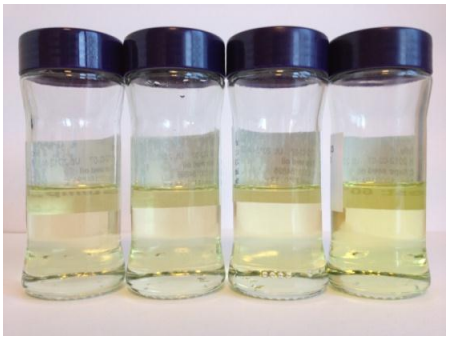

Figure 4.3: Rapeseed oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively. Notice the small change of colour of the sample stored at 60°C.

During the ASLT, the flavour and aroma of rapeseed oil was bland in storage at 22°C and 31°C. At the higher storage temperatures, rapeseed oil deteriorated to attributes of linseed oil. At ten weeks the character of this attribute had changed somewhat in both 40°C and 60°C, to a more painty perception, to become more round and less bitter at twelve weeks storage.

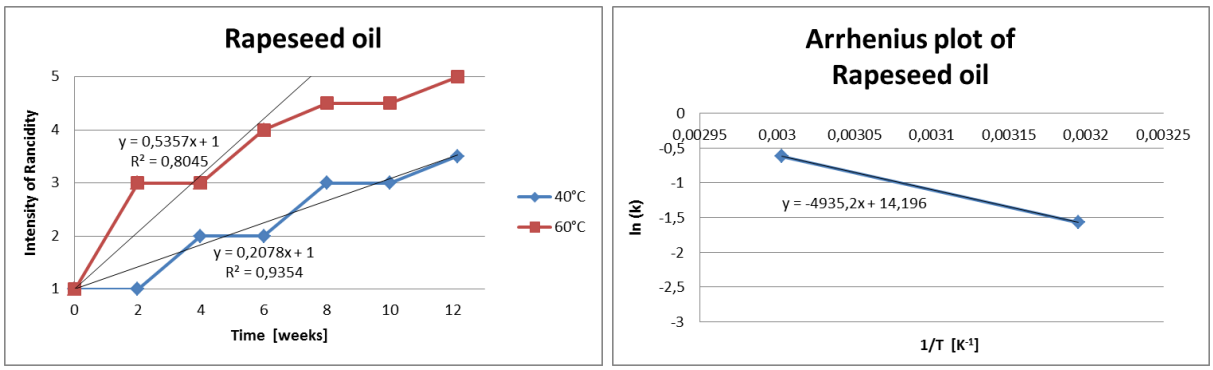

Figure 4.4: The intensity of rancidity of rapeseed oil plotted versus time (left) and Arrhenius plot of rapeseed oil (right).

The slope of the Arrhenius plot (figure 4.4) estimates the activation energy of rapeseed oil to 41 kJ/mol. The reaction rate constant at room temperature is approximated to 0.0794 kJ/mol K, making the reaction rate constants of 60°C and 40°C 6.7 and 2.6 times larger, respectively.

Rapeseed oil + antioxidant

Rapeseed oil with addition of antioxidant is liquid in all temperatures. It is not opaque and has slightly yellow colour (figure 4.5). During storage at 40°C and 60°C, the oil became more yellow but at twelve weeks storage the colour had faded.

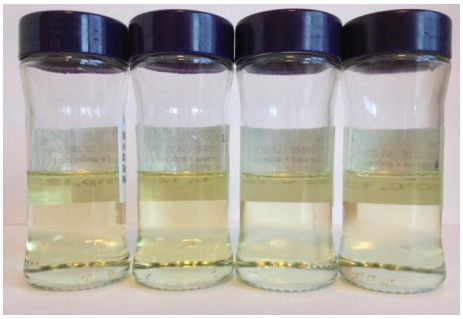

Figure 4.5: Rapeseed oil with addition of antioxidant stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively.

Rapeseed oil with addition of antioxidant has a bland aroma and flavour when stored twelve weeks in room temperature. At storage in 31°C there are very weak intensities of citrus or bloomy aroma, however not reaching to the second step of the intensity scale. During storage at 40°C and 60°C, the deterioration has a slightly nutty perception before the attributes changes to linseed oil. After six weeks storage at the highest temperature, the character of the oil becomes more painty, to increase in intensity and become very sharp at ten weeks. At twelve weeks the intensity of linseed oil had decreased considerably. This could be explained by the complex reactions of compounds formed and decomposed during lipid oxidation.

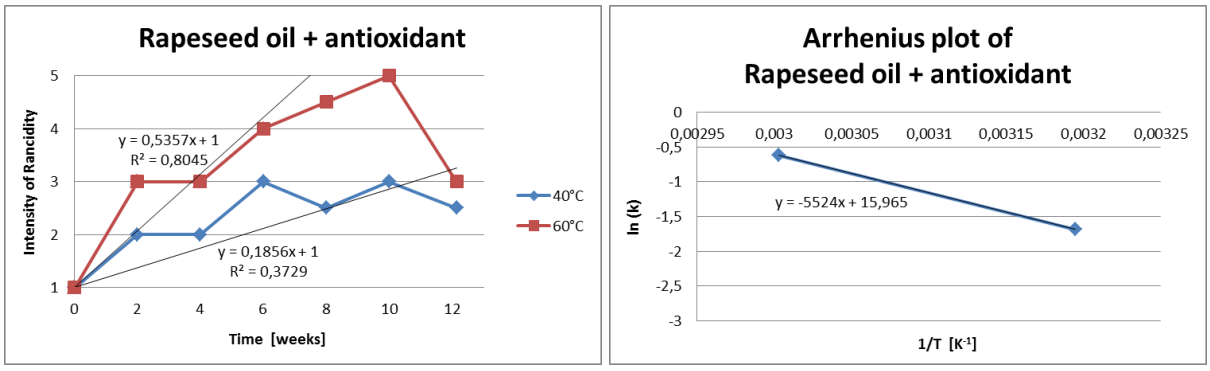

Figure 4.6: The intensity of rancidity of rapeseed oil with addition of antioxidant plotted versus time (left). Arrhenius plot of rapeseed oil with addition of antioxidant (right).

The Arrhenius plot of rapeseed oil mixed with antioxidant (figure 4.6) enables approximation of the activation energy of this oil to 46 kJ/mol. The reaction rate constant at room temperature is calculated to 0.0633 kJ/mol K. The rate constants of 60°C and 40°C are 8.5 and 2.9 times greater, respectively.

High oleic rapeseed oil

High oleic rapeseed oil is liquid in all temperatures, it is transparent and slightly yellow, as can be seen in figure 4.7.

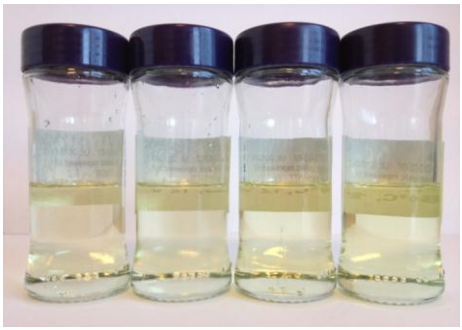

Figure 4.7: High oleic rapeseed oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively.

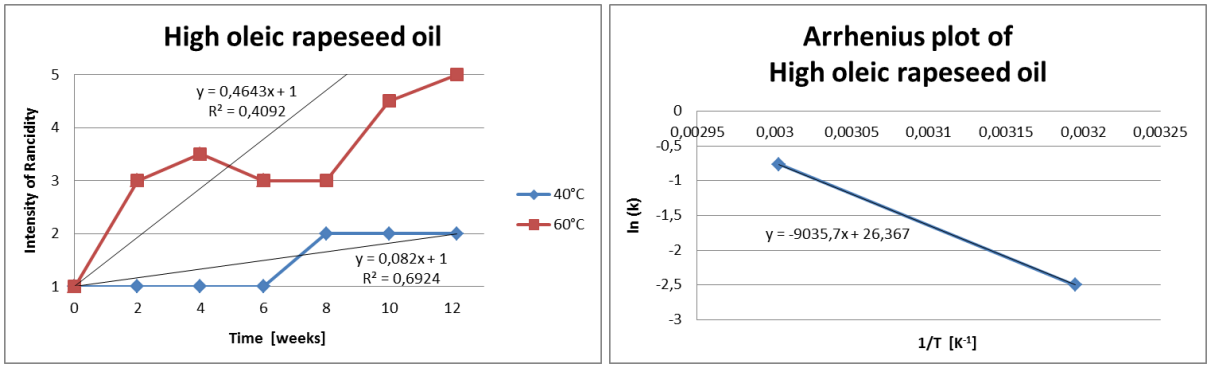

Figure 4.8: The intensity of rancidity of high oleic rapeseed oil plotted versus time (left) and Arrhenius plot of high oleic rapeseed oil (right).

The aroma and flavour of high oleic rapeseed oil is bland at twelve weeks storage in room temperature and 31°C. During storage at 40°C, the oil deteriorates with some perception of citrus flavour before the attribute of both aroma and flavour turns to linseed oil. At 60°C the attribute of linseed oil has a somewhat bitter character.

The activation energy of high oleic rapeseed oil, obtained from the slope of the Arrhenius plot in figure 4.8, is approximated to 75 kJ/mol. Extrapolation estimates the reaction rate constant of 22°C to 0.0141 kJ/mol K. The reaction rate constants of 60°C and 40°C are 33 and 5.8 times larger compared to this value, respectively. The high activation energy indicates that high oleic rapeseed oil is temperature dependent, which can be seen in the large difference between the reaction rate constants at different temperatures.

High oleic rapeseed oil + antioxidant

In conformity with the other rapeseed oils, high oleic rapeseed oil with addition of antioxidant is transparent and liquid in all temperatures. The colour is slightly yellow (figure 4.9). During storage at 60°C, the colour became more yellow with time, but the intensity had faded after twelve weeks.

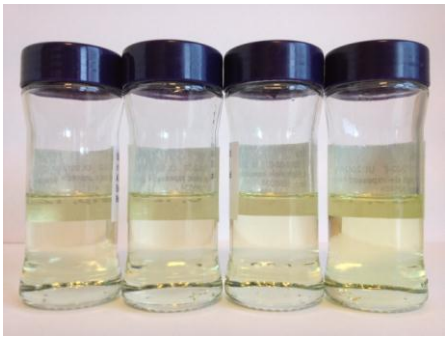

Figure 4.9: High oleic rapeseed oil with addition of antioxidant stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively.

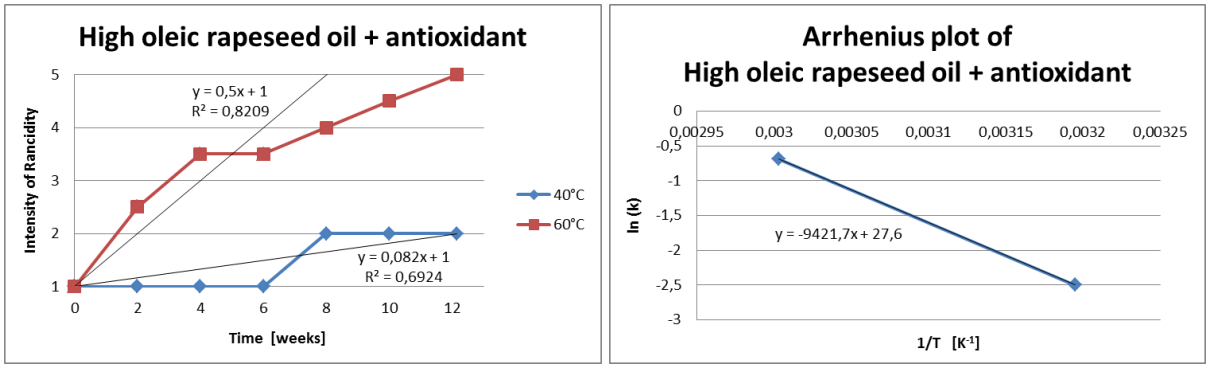

Figure 4.10: The intensity of rancidity of high oleic rapeseed oil with addition of antioxidant plotted versus time (left). Arrhenius plot of high oleic rapeseed oil with addition of antioxidant (right).

At room temperature and 31°C, high oleic rapeseed oil with addition of antioxidant has bland aroma and flavour. At 40°C and 60°C the rancidity has the attribute of linseed oil for both aroma and flavour. After eight weeks storage at 60°C the oil change character to become more painty, still with the attribute of linseed oil.

From the Arrhenius plot (figure 4.10) the activation energy of high oleic rapeseed oil mixed with antioxidant is estimated to 78 kJ/mol. The rate constant of the deterioration reaction occurring in room temperature is approximated by extrapolation to 0.0131 kJ/mol K. Compared to the reaction rate constants of 60°C and 40°C, this value is 38.3 and 6.3 times smaller, respectively. The large difference in k-values and the high activation energy indicate a high temperature dependence.

Sunflower oil

Sunflower oil is transparent and liquid in all temperatures and the colour is slightly yellow, as can be seen in figure 4.11.

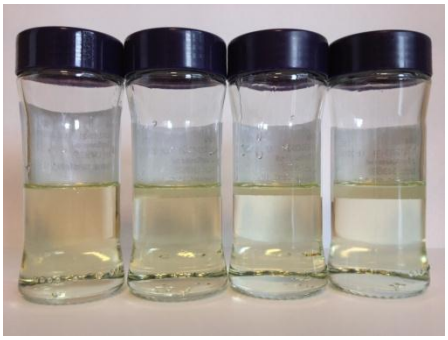

Figure 4.11: Sunflower oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively.

The flavour and aroma of sunflower oil is bland during storage at the lower temperatures. At 40°C the arising attributes are linseed oil and cardboard and at 60°C the linseed oil has a slightly bitter and qualmy character.

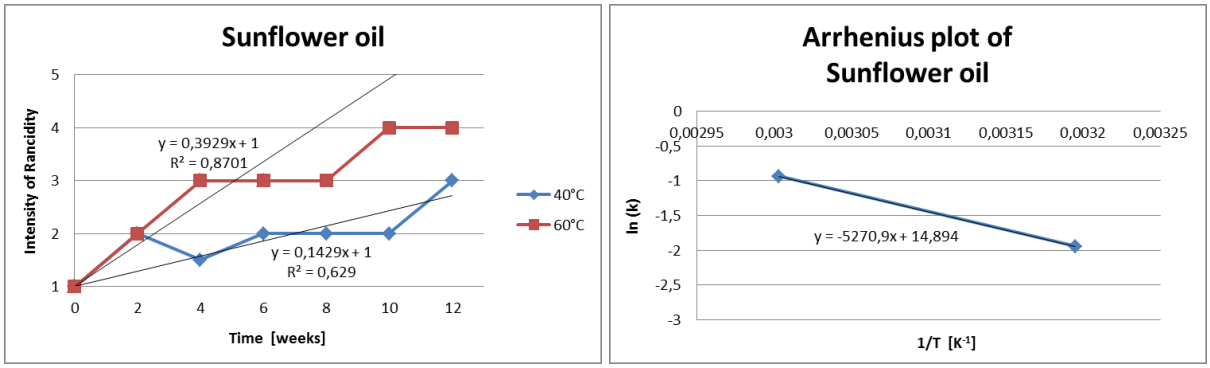

Figure 4.12: The intensity of rancidity of sunflower oil plotted versus time (left) and Arrhenius plot of sunflower oil (right).

The activation energy of sunflower oil is calculated to 44 kJ/mol from the Arrhenius relation shown in figure 4.12. The reaction rate constants of 60°C and 40°C is approximated to 0.3929 kJ/mol K and 0.1429 kJ/mol K, respectively, being about 7.7 and 2.8 times greater than the estimated reaction rate constant of 22°C approximated to 0.0511 kJ/mol K.

High oleic sunflower oil

High oleic sunflower oil is liquid in all temperatures and slightly yellow in colour. It is somewhat opaque at 22°C, 31°C and 40°C, and transparent at 60°C (figure 4.13). This is due to a natural presence of waxes that has not been removed during the refining processes, which melt at higher temperatures (Bringsarve, 2012).

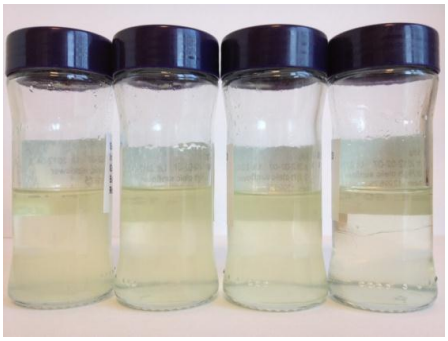

Figure 4.13: High oleic sunflower oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively. Notice the difference in opacity between samples.

High oleic sunflower oil is bland at storage in low temperatures and deteriorates with the formation of aroma and flavour of cardboard, citrus and linseed oil at 40°C, whereas at 60°C the attribute perceived is linseed oil with varied intensity.

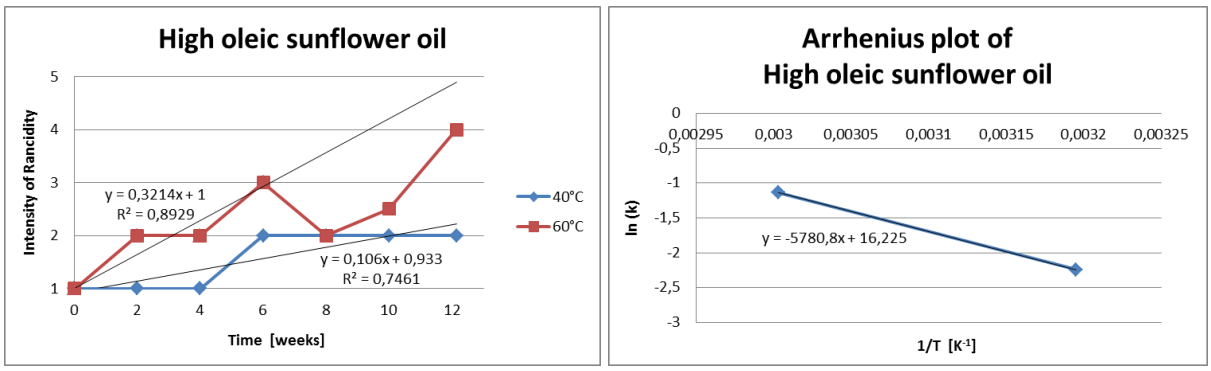

Figure 4.14: The intensity of rancidity of high oleic sunflower oil plotted versus time (left) and Arrhenius plot of high oleic sunflower oil (right).

From the Arrhenius plot seen in figure 4.14, the activation energy of high oleic sunflower oil is estimated to 48 kJ/mol and the reaction rate constant of 22°C is approximated to 0.0344 kJ/mol K. The rate constants for the reactions occurring at 60°C and 40°C (figure 4.14) are calculated to be 9.4 and 3.1 times greater compared to room temperature, respectively.

Rapeseed + sunflower oil

The mixture of rapeseed and sunflower oil is liquid at all temperatures. The colour is slightly yellow, and in storage at 22°C and 31°C the mixture is slightly opaque, as a result of the presence of waxes, see figure 4.15. In storage at 60°C, the oil became slightly more yellow, but the colour faded again after ten weeks storage.

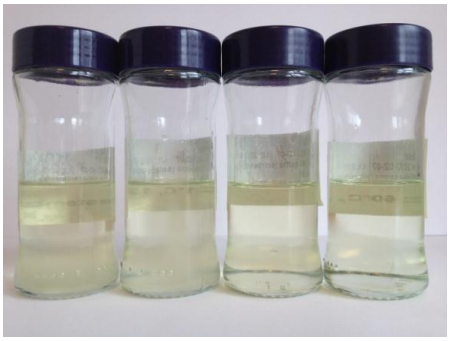

Figure 4.15: The mixture of rapeseed and sunflower oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively.

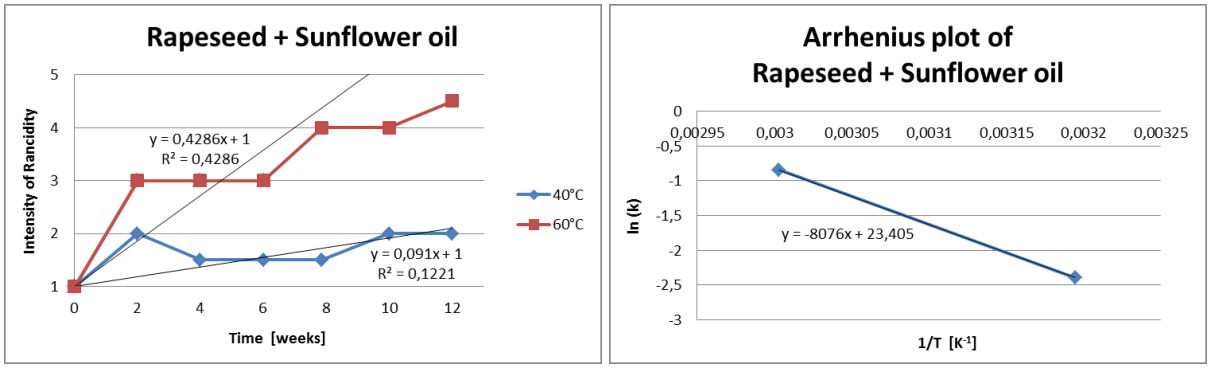

Figure 4.16: The intensity of rancidity of the mixture of rapeseed and sunflower oil plotted versus time (left). Arrhenius plot of the mixture of rapeseed and sunflower oil (right).

The oil mixture of rapeseed and sunflower oil has bland flavour and aroma at room temperature and 31°C. At 40°C the deterioration is perceived as linseed oil and cardboard, whereas at 60°C the attribute of linseed oil is appearing with increased intensity, being of bitter character.

The activation energy of the mixture of rapeseed and sunflower oil is calculated to 67 kJ/mol from the Arrhenius plot (figure 4.16). The reaction rate constant at room temperature is estimated to 0.0188 kJ/mol K, and the reaction rate constants of 60°C and 40°C are 22.7 and 4.8 times greater than this, respectively.

Coconut oil

Coconut oil is semi-solid and strongly opaque at room temperature. During storage at 31°C, 40°C and 60°C, coconut oil is liquid and transparent, which is visualised in figure 4.17.

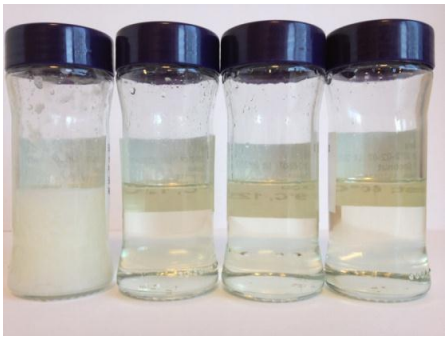

Figure 4.17: Coconut oil stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively. Note the change of state between storage at room temperature and 31°C.

Coconut oil has a slight flavour and aroma of citrus during storage at the lower temperatures, but the intensity does not reach the grade "weak" on the scale. In storage at 40°C and 60°C, the perception of citrus is mixed with cardboard and linseed oil, to develop into only linseed oil after eight weeks at 60°C.

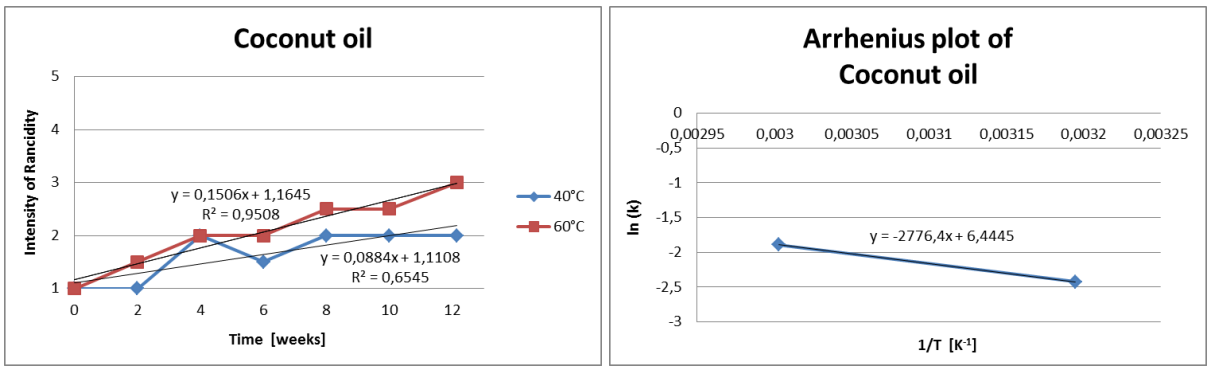

Figure 4.18: The intensity of rancidity of coconut oil plotted versus time (left) and Arrhenius plot of coconut oil (right).

The Arrhenius relation of coconut oil is plotted in figure 4.18. From the slope, the activation energy of the oil is calculated to 23 kJ/mol. Extrapolation obtains an approximated value of the reaction rate constant at room temperature to 0.0515 kJ/mol K. The values of the rate constants for reactions in 60°C and 40°C are 2.9 and 1.7 times greater compared to this value, respectively. The low activation energy indicates that lipid oxidation of coconut oil is not influenced by temperature.

Shea stearin

Shea stearin is solid at 22°C, 31°C and 40°C, hence being completely opaque, not yellow. At 40°C the samples are somewhat melted and at 60°C shea stearin becomes liquid and transparent (figure 4.19). However, there are small remains of cloudily fat crystals. The colour is not yellow at all.

Figure 4.19: Shea stearin stored twelve weeks at room temperature (22°C), 31°C, 40°C and 60°C, from left to right, respectively. The change of state occurs at storage between 40°C and 60°C, due to the high melting point.

Shea stearin has a basic aroma and flavour of cardboard and stearin at 22°C and 31°C. At 40°C and 60°C the intensity of these attributes increases and the oil also change perception to linseed oil.

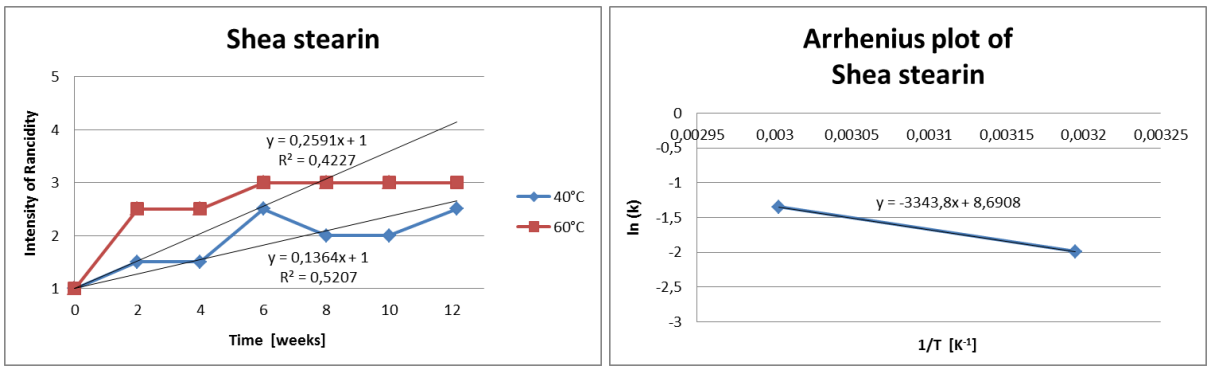

Figure 4.20: The intensity of rancidity of shea stearin plotted versus time (left) and Arrhenius plot of shea stearin (right).

The slope of the Arrhenius plot (figure 4.20) yields an approximation of the activation energy of shea stearin to 28 kJ/mol. The rate constant of the reaction occurring in room temperature is extrapolated to be 0.0711 kJ/mol K. The rate constants of 60°C and 40°C are 3.6 and 1.9 times larger than this value, respectively.

4.1.2 Comparative Discussion

To visualise the variation of reaction rate constants of each oil and temperature obtained from the sensory evaluation, they are summarised in table 4.2. The extrapolated reaction rate constants of room temperature are also listed, as well as the approximated relationship of the magnitude between the k-values.

Table 4.2: The reaction rate constants of 60°C and 40°C obtained from the sensory evaluation of oils included in the accelerated stability test, and the corresponding value at room temperature, extrapolated from the Arrhenius relation. The relationships of the magnitude of the reaction rate constants are also listed.

Sample	$\mathbf{k_{60°C}}$ [kJ/mol K]	$\mathbf{k_{40°C}}$ [kJ/mol K]	$\mathbf{k_{22°C}}$ [kJ/mol K]	$\mathbf{k_{60°C}: k_{40°C}: k_{22°C}}$ Relationship
Palm oil	0.2647	0.1325	0.0656	4.0 : 2.0 : 1
Rapeseed oil	0.5357	0.2078	0.0794	6.7 : 2.6 : 1
Rapeseed oil + antioxidant	0.5357	0.1856	0.0633	8.5 : 2.9 : 1
High oleic rapeseed oil	0.4643	0.0820	0.0141	33.0 : 5.8 : 1
High oleic rapeseed oil + antioxidant	0.5000	0.0820	0.0131	38.3 : 6.3 : 1
Sunflower oil	0.3929	0.1429	0.0511	7.7 : 2.8 : 1
High oleic sunflower oil	0.3214	0.1060	0.0344	9.4 : 3.1 : 1
Rapeseed + sunflower oil	0.4286	0.0910	0.0188	22.7 : 4.8 : 1
Coconut oil	0.1506	0.0884	0.0515	2.9 : 1.7 : 1
Shea stearin	0.2591	0.1364	0.0711	3.6 : 1.9 : 1

The reaction rate constants are estimated by trend lines from the result of the sensory evaluation. The correlations of these trend lines vary and are in some cases not good, indicating that evaluation of samples could have been performed more frequently. However, this would not have been possible due to the large amount of samples needed to evaluate the stability of ten different oils. In order to obtain the best correlation showing the tendency of formation of rancidity, some data points were excluded when making the trend lines.

Johansson (1976) assume that the speed of oxidation is doubled by each 15°C increase in temperature within the 20-60°C interval, and Kristott (2000) claims that the rate of oxidation reactions is roughly doubled per 10 K temperature increase. The results of all oils except three included in the stability tests end up somewhere in between these two indications of doubled oxidation rate with increased temperature. These three oils; pure high oleic rapeseed oil, high oleic rapeseed oil with addition of antioxidant and the mixture of rapeseed and sunflower oil, all have high activation energies, indicating a high temperature dependence, which could explain this difference from the other oils. As presented previously in the chapter, there is a wide range of the activation energies obtained for each oil, which are presented in table 4.3.

Table 4.3: List of the activation energies for each vegetable oil, calculated from the corresponding Arrhenius plots.

Sample	$\mathbf{E_A}$ [kJ/mol]
Palm oil	30
Rapeseed oil	41
Rapeseed oil + antioxidant	46
High oleic rapeseed oil	75
High oleic rapeseed oil + antioxidant	78
Sunflower oil	44
High oleic sunflower oil	48
Rapeseed + sunflower oil	67
Coconut oil	23
Shea stearin	28

The wide range of activation energies can be explained by that they are based on the reaction rate constants of the temperatures included in the stability test. If there is a large difference between the rate constants of the two temperatures, the slope of the Arrhenius plot is large, making the activation energy of the oil high. This is an indication that the oil is temperature dependent, and that elevated temperature is demanded to increase the rate of the deterioration of the oil. If the difference of the reaction rate constants of 60°C and 40°C is small, the slope of the Arrhenius plot is small, hence the estimation of the activation energy is small. As previously mentioned, pure high oleic rapeseed oil, high oleic rapeseed oil with addition of antioxidant and the mixture of rapeseed and sunflower oil have high activation energies, as can be seen in table 4.3. The reason for this is that the intensity of rancidity increases much more during storage at 60°C compared to at 40°C, and this indicates that these oils are temperature dependent, hence highly affected by a change in temperature. This explains the wide range in relationship of reaction rate constants (table 4.2), when an increase from room temperature to 60°C significantly influence the deterioration of the oil and formation of rancidity.

The intensity of rancidity varies among the different vegetable oils tested. The main reason for this is that the fatty acid composition varies between the different oils. Coconut oil and shea stearin, having a very high amount of saturated fatty acids (see table 2.3) were expected to be stable against rancidity. Also palm oil, consisting of mainly saturated fatty acids, was expected to be relatively stable. When comparing the results of the sensory evaluation performed for each oil included in the accelerated stability test, coconut oil, shea stearin and palm oil were shown to have the least degree of rancidity and also among the smallest reaction rate constants of 60°C and 40°C (table 4.2). This results in that these three oils have low activation energies (table 4.3), indicating that an increase in storage temperature does not affect the deterioration of the oil. Rapeseed oil was shown to be the least stable oil with the highest perception of rancidity in both 60°C and 40°C, and considering the fatty acid composition of almost exclusively unsaturated fatty acids with a high amount of polyunsaturated fatty acids, this was expected. The rapeseed oil with addition of antioxidant showed similar deterioration at 60°C compared to pure rapeseed oil, but had somewhat less

rancid intensity during storage at 40°C. This indicates that the antioxidant has a slight effect on delaying the oxidation. Pure high oleic rapeseed oil and high oleic rapeseed oil mixed with antioxidant were shown to have very similar deterioration and intensities of rancidity, although the attributes were slightly different, being more bitter or painty respectively. This indicates that the antioxidant has no major influence on the high oleic rapeseed oil. Compared to pure rapeseed oil, high oleic rapeseed oil deteriorated with somewhat less reaction rate, at least during 40°C. This was predicted due to the higher amount of polyunsaturated fatty acids of the pure rapeseed oil, however the changes were expected to be larger. Sunflower oil was shown to be more stable than rapeseed oil, in accordance with the fatty acid composition. Sunflower oil consists of no polyunsaturated fatty acids while the amount in rapeseed oil is 10 %. High oleic sunflower oil reached the same intensity of rancidity during 40°C as pure sunflower oil. However, high oleic sunflower oil was shown to be somewhat more stable during storage at 60°C, indicating that the large reduction in polyunsaturated fatty acids makes this oil less prone to oxidation. The mixture of rapeseed and sunflower oil had a deterioration of between that of unmixed rapeseed oil and sunflower oil, in agreement with the fatty acid composition.

AarhusKarlshamn AB evaluates oils based on the awareness that the perception of rancid rapeseed oil is linseed oil; rancid palm oil has the attribute of cardboard; sunflower oil becomes qualmy and greasy when rancid, and that coconut oil becomes soapy and sharp at deterioration (Bringsarve, 2012). The sensory evaluation performed of the oils included in this stability test has shown mostly the same tendencies. All four varieties of rapeseed oil deteriorated with the attribute of linseed oil, and the perception of rancid palm oil was cardboard turning to linseed oil. However, the sunflower oils deteriorated with the formation of attributes of cardboard and linseed oil, with a slightly bitter and with time also qualmy character. Perhaps the attributes perceived from sunflower oil by the panel at Santa Maria AB would have been different and more similar the ones explained by AarhusKarlshamn AB, with more training of reference tests to describe rancid samples of sunflower oil. The coconut oil was perceived as influences of citrus mixed with cardboard and linseed oil, and not soapy at all. This is probably due to that the soapy character of rancid coconut oil arises from hydrolysis, and would be more likely to occur in food products than pure oil during this relatively short storage time.

The reason for some of the rapeseed oils changing colour to become more yellow with time during storage at high temperatures, could be that the complex chemical reactions decomposing and forming several different compounds also form compounds with different intensities of yellowness. The observation that some of these oils lost their yellow colour with time is somewhat more difficult to explain, but could probably also be due to the complexity of the chemical reactions taking place at the deterioration of a vegetable oil.

Due to that only two temperatures could be used to obtain the Arrhenius plots, the accuracy of the results could be improved. This could be made by changing the experimental setup in two different ways. The first is to continue the storage at the lower temperatures 22°C and 31°C for a longer time, until the deterioration occurs at these conditions as well. The other suggestion, which is recommended in order to save time, is to include several testing conditions with elevated temperatures. Temperatures suitable for stability tests of vegetable oils would be 40°C, 45°C, 50°C, 55°C and 60°C. However, it is problematic to practically perform a test like that, due to that several different heating chambers must be used simultaneously.

4.2.3 Measurement of Peroxide Value and *para*-Anisidine Value

The results from the analysis of PV and AV of samples of palm oil and rapeseed oil are listed in table 4.4. They show that the PV is rather stable for both palm oil and rapeseed oil stored at room temperature, with rapeseed oil having a slight increase. The fact that the PV varies up and down compared to the reference stored in refrigerator at 4°C from delivery until analysis, could be due to that the analysis is made only on single samples. In order to achieve more significant numbers, duplicates or rather even more number of samples should be analysed for each oil and storage condition. However, the results presented here indicate that both palm oil and rapeseed oil are stable at room temperature and that the deterioration has not yet started, hence being in the lag phase. The same indication is given by the AV, being low for both oils. These results are comparable with the sensory analysis showing that no deterioration occurred at storage in room temperature.

Table 4.4: Peroxide values and *para*-anisidine values of samples of palm oil (left) and rapeseed oil (right) stored at different conditions. The reference samples were stored in refrigerator at 4°C from delivery until analysis.

Sample		PV [meq/kg]	AV
Palm oil	Reference	1.4	0.5
Palm oil	2 weeks, 22°C	1.8	
Palm oil	4 weeks, 22°C	1.3	
Palm oil	6 weeks, 22°C	1.3	
Palm oil	8 weeks, 22°C	1.5	1.6
Palm oil	2 weeks, 40°C	2.1	
Palm oil	4 weeks, 40°C	2.6	
Palm oil	6 weeks, 40°C	4.1	
Palm oil	8 weeks, 40°C	6.7	1.7

Sample		PV [meq/kg]	AV
Rapeseed oil	Reference	1.5	0.6
Rapeseed oil	2 weeks, 22°C	1.3	
Rapeseed oil	4 weeks, 22°C	1.3	
Rapeseed oil	6 weeks, 22°C	1.8	
Rapeseed oil	8 weeks, 22°C	2.2	0.7
Rapeseed oil	2 weeks, 40°C	2.6	
Rapeseed oil	4 weeks, 40°C	16	
Rapeseed oil	6 weeks, 40°C	33	
Rapeseed oil	8 weeks, 40°C	45	9.0

The samples of palm oil stored at 40°C have an increase in PV indicating a slight deterioration. The AV, measuring secondary oxidation products giving rise to aroma and flavour, is the same for palm oil stored 8 weeks at 40°C as during storage at room temperature. The AV was expected to be somewhat higher for the sample stored at 40°C, since this sample was scored 3.5 at the five grade rancidity scale of the sensory evaluation. However, the increase in PV and AV indicates a slight deterioration of palm oil which was observed during the sensory evaluation. The AV of the samples of rapeseed oil stored at 40°C is multiplied compared to storage at room temperature and the PV has increased exponentially. These numbers indicate that the reaction is autocatalysing itself and that some of the primary oxidation products have converted to secondary oxidation products, demonstrating that the exponential phase of lipid oxidation is entered. This was detected also at the sensory evaluation when the rancidity was scored as 4.5 on the five grade scale. Hence, these results show that rapeseed oil is less stable compared to palm oil, as expected from the fatty acid composition.

4.2.4 Selection of Oils for Further Evaluation

Among the vegetable oils investigated in the stability test, rapeseed oil and sunflower oil were selected to be evaluated in the four spice mixtures. These two oils were not shown to have the

best temperature stability, therefore they are best to be used at low temperatures. Spice mixtures produced at Santa Maria AB are intended to be stored in a cool and dry place, not above room temperature, hence it was concluded that there is no need for use of a high stability oil as processing aid when manufacturing spice mixtures. This statement was additionally substantiated when it was found that the spice factory of Santa Maria AB in Saue, Estonia, use rapeseed oil as processing aid in the spice mixtures produced there. Another aspect of selecting proper oil as replacement for palm oil is that it might be better to have an oil being liquid at room temperature. There is a risk that the heat treatment of palm oil in the production process induces some deterioration of the oil prior the addition to the spice mixtures. The elevated temperature could also affect the ingredients in the spice mixture in a negative way, for example by affecting the components of biological origin being responsible for colour and flavour.

Rapeseed oil and sunflower oil were also selected due to their low price and easy handling. Due to that the oil used as processing aid are consumed in relatively small amounts, it is important that the oil can be delivered in small quantities with short intervals. Sunflower oil and rapeseed oil can be provided in several different amounts (Blomdahl, 2012).

4.3 Spice Mixtures

The four different spice mixtures produced with and without addition of oil are shown in figure 4.21. The effect of adding oil in the production process is clear when looking at Honey Barbeque Seasoning. No difference in appearance of samples containing the three different oils could be detected for any of the seasonings.

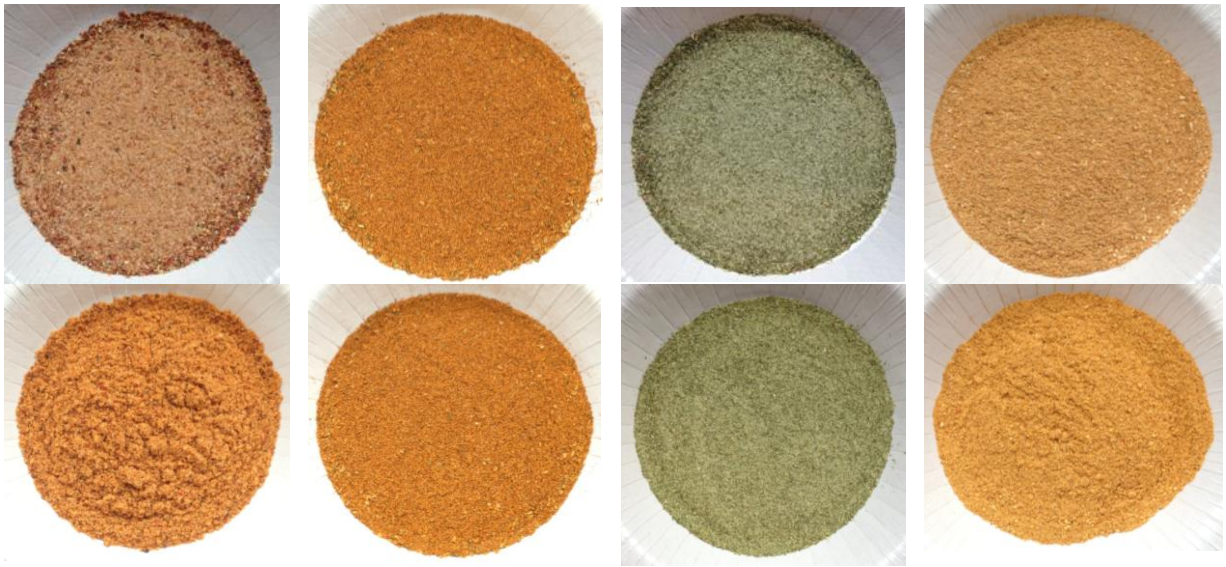

Figure 4.21: Barbeque Honey Seasoning, Fajita Spice Mix, Italian Salad Seasoning and Taco Spice Mix without addition of oil (above) and with oil added (below).

Evaluation of the spice mixture samples stored at 60°C during eight weeks showed that various seasonings react differently to high temperatures and that the temperature was too high when evaluating spices. All four spice mixtures became very dry and dark during storage, as can be seen in figure 4.22.

Figure 4.22: Barbeque Honey Seasoning, Fajita Spice Mix, Italian Salad Seasoning and Taco Spice Mix stored eight weeks at 60°C.

The samples of Barbeque Honey Seasoning were agglomerated and caked. The colour of the seasoning was very dark and brown, compared to samples stored at lower temperatures. The flavour had lost character to taste salt and stale honey with growing heat. The samples of Fajita Spice Mix had not changed much compared to samples stored at lower temperatures. The red colour and lustre was lost, but there were no agglomeration or caking. The flavour was salt and somewhat sweet with growing heat. The samples of Italian Salad Seasoning were similar to those of Barbeque Honey Seasoning in agglomeration and caking. The colour was darker and more brown compared to storage at low temperatures and the flavour had changed to taste almost only of salt, with no more than tendencies of herbs and onion. Taco Spice Mix was shown to agglomerate after just a few days at 60°C. After eight weeks storage the hardness of all caked samples was the same, and it was not possible to decide whether any samples were different from the others, however the samples without addition of oil could have been somewhat stiffer. The quick caking of Taco Spice Mix could be explained by that certain ingredients, such as yeast extract, melt at high temperatures. There was no difference in taste among the samples containing different oils but the flavour had changed to taste sweet, salt and cumin. No rancid taste was detectable among any of the samples stored eight weeks at 60°C.

4.3.1 Analysis of Angle of Repose

Angle of repose measurements were made on all four spice mixtures at storage in room temperature and 40°C after four and eight weeks. Due to the seasonings stored in glass jars being agglomerated very early, Barbeque Honey Seasoning was additionally evaluated after two and six weeks at storage in 40°C, and Italian Salad Seasoning was additionally evaluated after six weeks. During storage at 60°C, the seasonings stored in glass jars became very agglomerated and dry, therefore being unsuitable for angle of repose measurements. These spice mixtures were shown to be quite free-flowing when mixed to reduce the clumps, because of the dryness of the powder. Therefore it was concluded to be inappropriate to perform angle of repose measurements as an indication on clump formation and cohesiveness for these two spice blends stored at 60°C. Taco Spice Mix was found to cake after just a few days in 60°C, hence neither these samples were a part of the angle of repose analysis.

To make the cohesive powders Barbeque Honey Seasoning, Taco Spice Mix and the test mixture pour through the funnel, vibrations were obtained by gently tapping on the top of the edges of the funnel. The number of taps was counted, but no correlations of the added oil and the number of taps demanded could be made for any of the spice mixtures or oils. Italian Salad Seasoning and Fajita Spice Mix were free-flowing enough at all storage conditions not to need the vibrations obtained by tapping.

The size of angles of repose varied a lot both between the different spice mixtures and within each seasoning. There is a variation between the samples without added oil and with the sample containing oil. The most significant example of this is the samples of Barbeque Honey Seasoning, where all samples without addition of oil are significantly different from all samples containing oil, which can be seen in figure 4.23. This indicates that the amount of added oil affects the cohesiveness of the powder. Note that it is the mean value and the standard deviation of each mean value of angle of repose that is shown in all the figures regarding angle of repose.

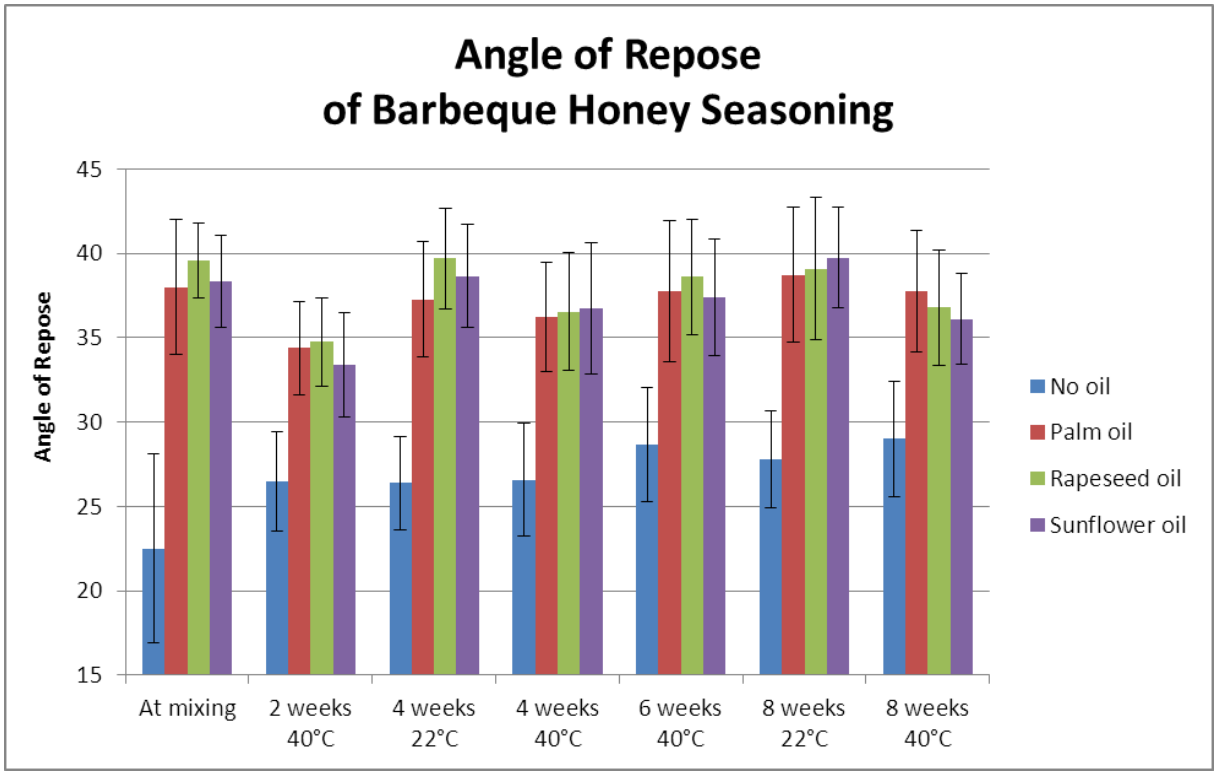

Figure 4.23: Results of angle of repose measurements of samples of Barbeque Honey Seasoning stored at different conditions.

Comparison of the angles of repose of the three different oils within each storage condition show that the angles are similar and not differ from each other. This is true for all four spice mixtures, but the angles of repose vary with different magnitudes, see figures 4.23, 4.24, 4.25 and 4.26. Note that the scales on the y-axes are not the same.

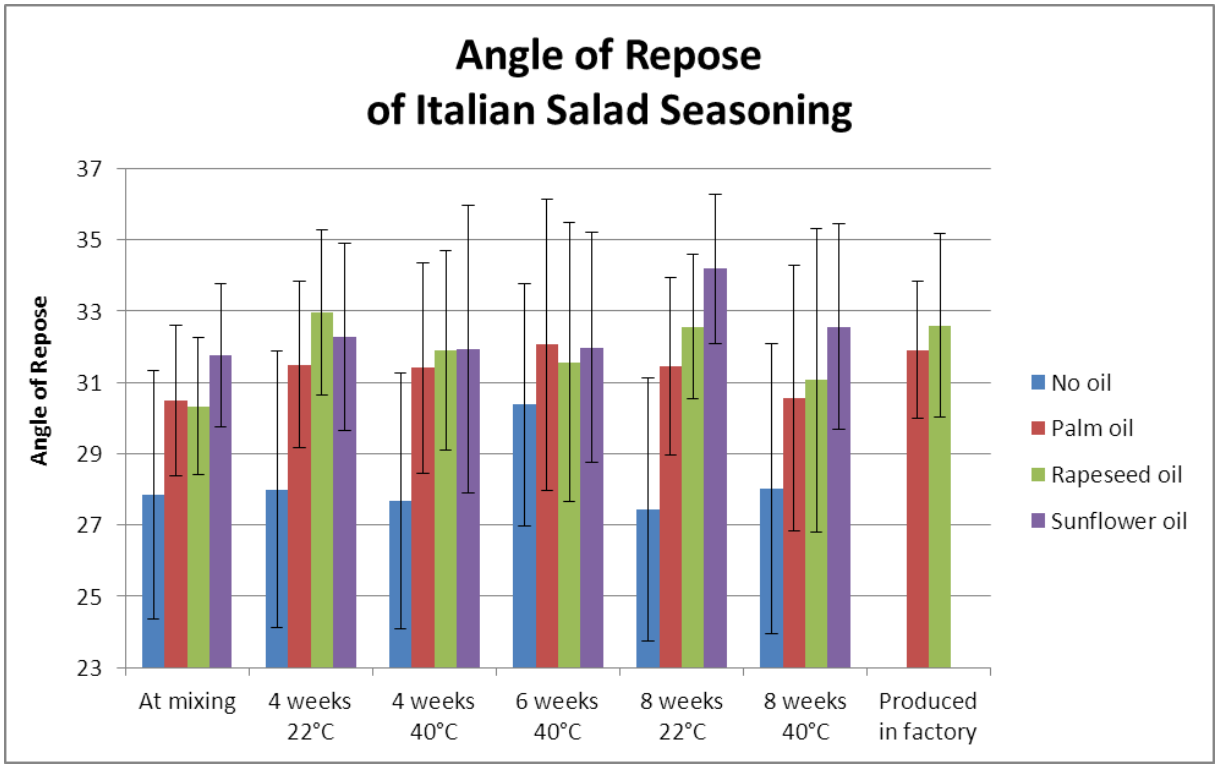

Figure 4.24: Results of angle of repose measurements of samples of Italian Salad Seasoning stored at different conditions.

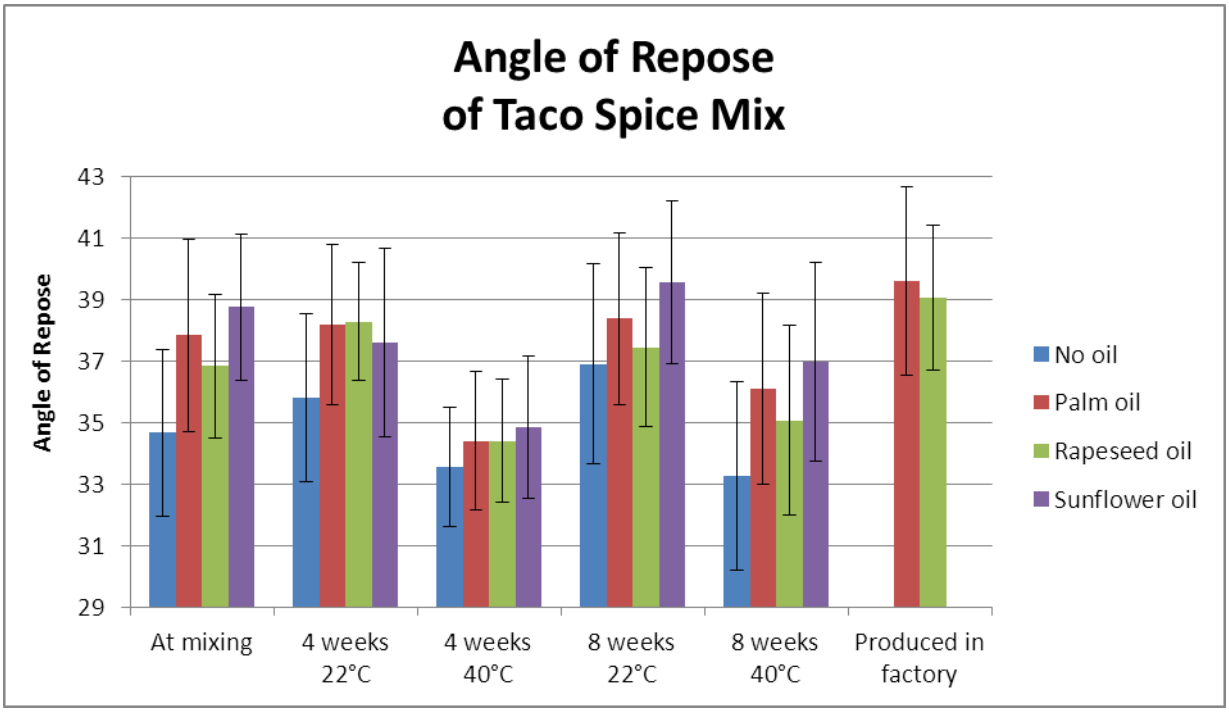

Figure 4.25: Results of angle of repose measurements of samples of Taco Spice Mix stored at different conditions.

A possible explanation for the variety of magnitude of the angle of repose during different storage conditions, as can be seen for Taco Spice Mix (figure 4.25), would be that the

surrounding temperature changes the properties of the powder. Storage at 40°C could make the spice mixture somewhat dryer compared to storage at room temperature, thereby making it less cohesive, explaining the slight reduction of angle of repose. However, this explanation does not hold for Fajita Spice Mix (figure 4.26), where the angles of repose are somewhat increased for all samples stored eight weeks, irrespective of the storage temperature. This behaviour could be due to that these two spice mixtures contain different ingredients that react to storage in different ways. Generally, the angles of repose of Taco Spice Mix are larger compared to Fajita Spice Mix, showing that the latter seasoning is less cohesive. The angles of repose for almost all samples of the four spice mixtures containing the three different oils are in the range of 30 to 45°, indicating some cohesiveness. If following the classification of angles of repose of 40°, most samples are classified as free-flowing.

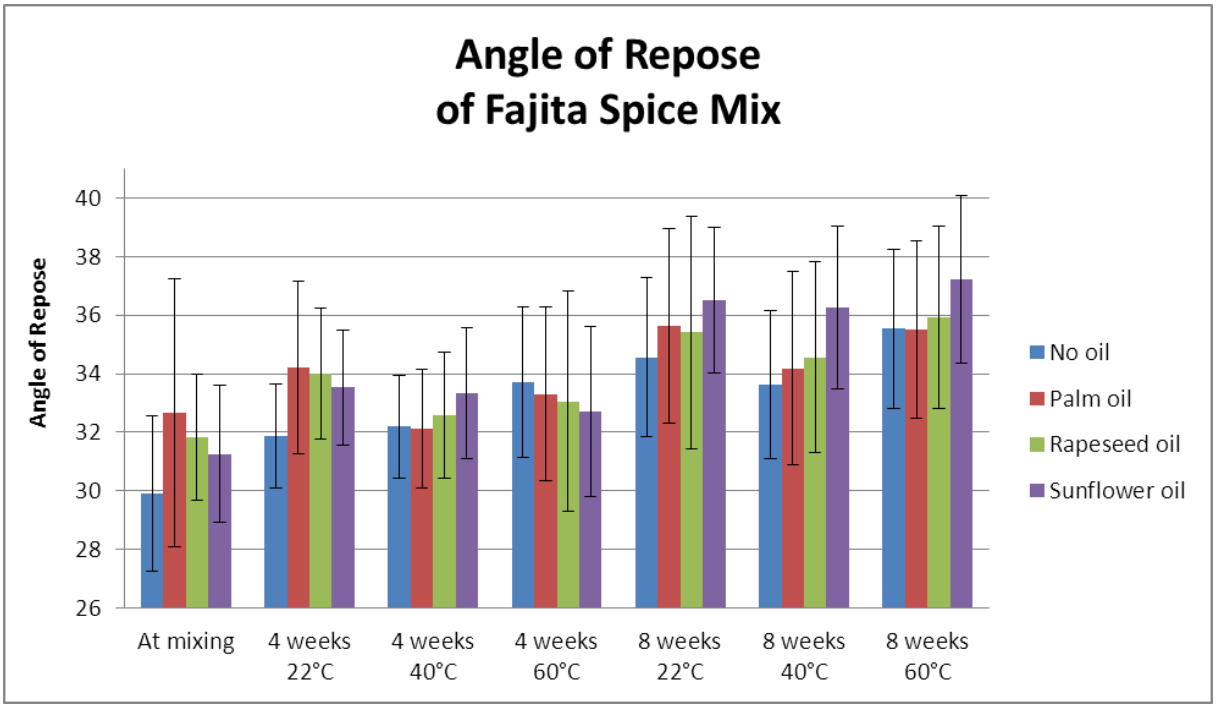

Figure 4.26: Results of angle of repose measurements of samples of Fajita Spice Mix stored at different conditions.

The spice mixtures produced at the test run in the factory were also analysed by angle of repose (see figures 4.24 and 4.25). The results indicate that the angle of repose of Italian Salad Seasoning produced with both palm oil and rapeseed oil are similar to the rest of the results. Taco Spice Mix with addition of palm oil and rapeseed oil, produced in the factory, have somewhat larger angles of repose compared to earlier results of different storage conditions, as can be seen in figure 4.25. This could be due to that they were manufactured later in the year when the relative humidity were higher, making the samples somewhat more cohesive.

The results of the angles of repose of the test mixture, consisting of hygroscopic ingredients, mixed with eight of the oils included in the stability test can be seen in figure 4.27. At mixing, the test mixture containing sunflower oil had slightly lower angle of repose compared to the other samples. However, after four and eight weeks of storage, no difference between the oils could be detected. There was a slight tendency that the angle of repose increased with storage time. The samples without addition of oil are shown to have a smaller angle of repose

compared to samples containing oil. This indicates that the addition of oil has an influence on the cohesiveness of the spice mixture and the angle of repose, regardless of which oil that is used.

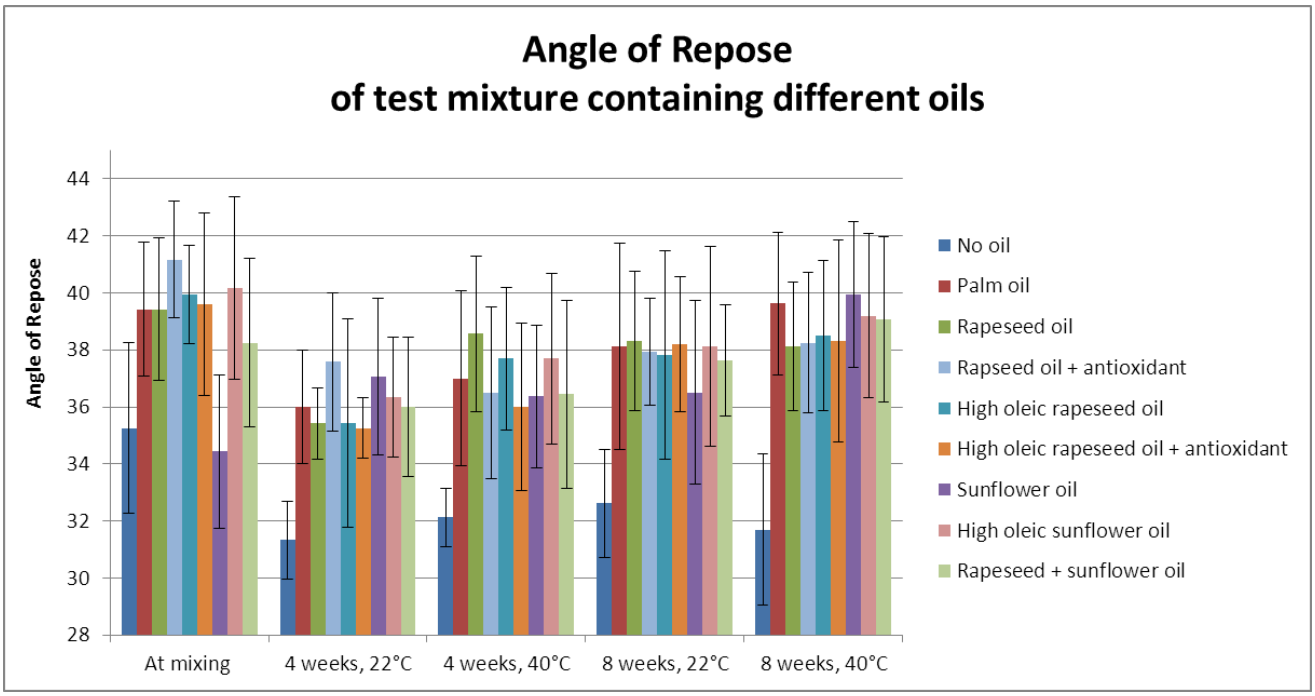

Figure 4.27: Results of angle of repose measurements of test mixture containing different oils stored at different conditions.

Conclusively, the evaluation of angle of repose gives no indication that any of the three oils selected to be investigated mixed with spice mixtures would have more or less influence on the agglomeration or caking of spice mixtures. However, the analysis of angle of repose was shown to be a time consuming method not giving any clear indications of the agglomeration of a sample.

4.3.2 Further Evaluation Methods

The visual evaluation of caking, dusting and separation of the four spice mixtures showed that there are differences between the different spice mixtures, but not depending of addition of different oils. The dusting was affected by the addition of oil to a large extent, especially for Barbeque Honey Seasoning. This seasoning was very dusty and separated without addition of oil, while the samples containing oil had almost no dust generation or separation. Instead the mixture was homogenous and less dusty, indicating that addition of oil as processing aid is necessary. However, the seasoning was flocculated, being an indication of that less oil could be used to give the best properties of the spice mixture. The samples of Italian Salad Seasoning showed the same behaviour of being dusty and separated without oil, but homogenous and less dusty when containing oil. There is not much difference of samples containing oil and samples without addition of oils of the seasonings Taco Spice Mix and Fajita Spice Mix, because these spice mixtures are relatively homogenous and does not generate dust to the same extent without addition of oil. However, the dust generation is somewhat decreased and the seasonings became even more homogenous with addition of oil. The samples of spice mixtures stored in glass jars at 40°C and 60°C were shown to be harder

and more agglomerated if no oil was added, compared to samples with addition of any of the three oils. For the other spice mixtures, there were no detectable differences in caking between samples containing oil or not. These visual results can be related to the results of the analysis of angle of repose, showing relatively large differences in angles of repose between spice mixtures containing oil compared to samples without addition of oil, especially for Italian Salad Seasoning and Barbeque Honey Seasoning.

All samples packed in laminate bags were weighed before and after storage. Almost all samples decreased in weight during storage. The decrease ranged from about 0.2 % for Taco Spice Mix stored at room temperature in four weeks, to about 5 % for Taco Spice Mix stored eight weeks at 60°C. The decrease was similar for all samples of the same seasoning stored at the same conditions, regardless of the type of oil or if oil was added at all, indicating that the change in weight had nothing to do with the oil but with the composition of the spice mixture and the storage conditions. One explanation for the weight decrease could be that the relative humidity was comparatively low at the time when the test was made. The samples increasing in weight during storage were the test mixture stored in room temperature. This could be due to the hygroscopic properties of the ingredients of the test mixture. The fact that the samples of test mixture stored at 40°C decreased in weight could be explained by that the fan in the heating chamber increased the exchange of air, removing more moisture.

The agglomeration and caking of spice mixtures is highly related to the relative humidity during storage. According to Lilliebjelke (2012) a spice mixture has no tendency of caking if it has not started to agglomerate after storage in laminate bags after eight weeks at 40°C. The storage tests performed in the present work did not show much caking after eight weeks, neither the test mixture agglomerated. The reason for this is most likely that the test session was performed during the winter and early spring, when the storage conditions are the best. The problems of caking are greater during the summer, when the agglomeration may be extensive. In February and March, the air humidity range between 10 % and 20 % relative humidity (RH) outside, and during the summer it may be as high as 80-90 % RH. In the spice plant the humidity in the air is controlled to make sure that there is maximum 40 % RH, in order to reduce agglomeration and other problems during manufacturing (Rice, 2012).

The test method performed to investigate a possible correlation between caking and the amount of spice mixture remaining in the bag after turning it upside down gave inconsistent results. The amount of spice mixture left in the bag after holding it upside down had no correlation to the extent of caking. Samples being free-flowing could stick in the bag, while samples containing agglomerates could easily pour out of the bag, and vice versa. Therefore it is not possible to make any conclusions about the extent of caking and how easily the content pours out of the laminate bag.

Seven different spice mixtures produced at Santa Maria AB spice plant in Saue, Estonia, were sensory evaluated. Newly produced samples were compared to products long passed the best-before date. The old samples had lost some of the flavour and colour; especially herbaceous flavours and green and red colours were faded. No rancid taste or aroma was detectable and none of the samples were agglomerated. Santa Maria AB have not received any reclamations or complaints from consumers about spice mixtures being rancid (Säfström, 2012; Tammel, 2012). To confirm that rancidity is not an issue in spice mixtures, samples of Taco Spice Mix and Barbeque Honey Seasoning were mixed with rancid rapeseed oil stored twelve weeks at 60°C, and cooked with minced meat and fried chicken, respectively. Sensory evaluation with comparison of freshly prepared samples showed that no rancid taste was detectable,

confirming the assumption. This could be explained by that the flavours of the ingredients of the spice mixture itself mask the rancid flavours.

4.3.3 Metal Ion Content

The result of the analysis of the presence of metal ions in the four spice mixtures are given in table 4.5. Iron was found in all seasonings while copper and manganese only were present in Fajita Spice Mix. Zinc was detected in all spice mixtures but Italian Salad Seasoning.

Table 4.5: The calculated amounts of the transition metal ions of copper, zinc, manganese and iron in the four spice mixtures. The line indicates no presence of ions.

Sample	**Cu** [µg/g dry weight]	**Zn** [µg/g dry weight]	**Mn** [µg/g dry weight]	**Fe** [µg/g dry weight]
Barbeque Honey Seasoning	-	5.02	-	46.44
Fajita Spice Mix	1.62	7.14	6.90	73.96
Italian Salad Seasoning	-	-	-	59.55
Taco Spice Mix	-	3.9	-	53.30

Reineccius (2006) states that a concentration of 0.3 ppm iron and 0.01 ppm copper is enough to produce a noticeable pro-oxidative effect. In comparison with these amounts, the presence of iron and copper could therefore be expected to induce oxidation in the spice mixtures, especially in Fajita Spice Mix. However, results presented in sections 4.2.4 and 4.3.2, indicate that rancidity of spice mixtures is no problem. The presence of transition metals most likely origin from the ingredients of the spice mixtures, and it would be interesting to further analyse the origin of specific components.

4.3.4 Test Run in Factory

The full-scale test run of Taco Spice Mix containing rapeseed oil as processing aid proceeded well. The dusting and agglomeration at the mixing step was not different from the ordinary production and the packaging on consumer laminate bags proceeded as usual. The personnel in the spice plant did not observe any deviations from the ordinary production, when palm oil is used as processing aid.

The full-scale test run of Italian Salad Seasoning with rapeseed oil was carried out with the same positive results as for Taco Spice Mix, with no detectable differences compared to manufacturing using palm oil as processing aid.

5 Conclusions and Future Work

The accelerated shelf life tests clearly showed that the stability of vegetable oils corresponds to the fatty acid composition and that oils composed of mainly saturated fatty acids were the most stable ones. These results were obtained despite that only two temperatures, 40°C and 60°C, were used in the Arrhenius plots, due to that all oils stored at the lower temperatures, 22°C and 31°C, were shown to be stable enough not to deteriorate with the perception of rancidity during twelve weeks storage. If performing similar stability tests again, the recommendations would be to include additional storage temperatures with smaller intervals between 40°C and 60°C, and to use room temperature as reference.

The method of evaluating the stability by sensory analysis was shown to be a good but difficult technique. More training should have been made prior to evaluation in order to coordinate the panel and increase the reproducibility of the outcome. It was shown to be easier to decide the intensity of rancidity rather than the actual attribute, especially when the intensity was low. Each oil was found to deteriorate with its own attributes, and an advice to Santa Maria AB is to extend the library of rancid attributes. It would have been interesting to analyse the deterioration of oils with TBARS that detect secondary oxidation products responsible for off-flavours, or to evaluate which molecules that are formed and decomposed during the lipid oxidation using gas chromatography coupled with mass spectroscopy, and to compare the intensity of rancidity with the results from such analysis.

Rapeseed oil was chosen to be the best replacement for palm oil as processing aid in the manufacturing process of dry spice mixtures. A good indication is that rapeseed oil is used in the spice factory of Santa Maria AB in Saue, Estonia, without any problems. Both the sensory evaluation and the analytical analysis of AV and PV performed, showed that rapeseed oil is not very stable at storage in elevated temperatures. However, at room temperature the oil was shown to be stable. In addition, the results evolved in this Master's Thesis have shown that rancidity in spice mixtures is no problem, and that rancid taste cannot be detected even in samples produced with rancid oil. Hence, there is no need to use a high stability oil as processing aid.

Analysis of angle of repose of four different spice mixtures produced with the addition of palm oil, rapeseed oil and sunflower oil respectively, compared with seasoning without addition of oil, showed no differences between spice mixtures containing different oils. However, after visual evaluation, it became clear that the addition of oil is necessary to make the spice mixture homogenous and to decrease the dust generation. The results also show that there are no detectable differences in agglomeration and caking between samples containing the three different oils.

The recommendation to Santa Maria AB is not to use the analysis of angle of repose to evaluate the agglomeration and caking of samples of spice mixtures. However, the method could be practical to use when deciding the amount of oil needed in a seasoning. Different spice mixtures demand different amount of oil to reduce dusting and to avoid separation of constituents of different density and size. To maintain the best properties of each spice mixture, it could be beneficial to revise the amount of oil in seasonings with different types of problems, such as dusting and caking, since it has been found that no addition of oil induce caking for some spice mixtures, and that too much oil added may make the powder very flocculent.

Another interesting investigation would be to compare the angles of repose of samples of spice mixtures stored at elevated temperatures, with samples stored longer period of time at room temperature. Hopefully this would give a correlation between the storage time in elevated temperature and the corresponding shelf life in room temperature. It would also be interesting to examine how other external factors affect the properties of spice mixtures, for example by varying the relative humidity instead of temperature during storage.

When performing accelerated tests to evaluate differences between spice mixtures, it was concluded that 40°C is a suitable storage temperature, as well as keeping samples at room temperature for comparison. Storage at 60°C induce too much reactions not occurring at ordinary storage during time, and therefore this temperature is not recommended when evaluating spice mixtures. Perhaps a lower temperature of 30°C or 35°C should be used instead.

In conclusion, the results of the present thesis work show that there was no difference between the oils investigated as processing aid in the manufacturing process of spice mixtures, but that the addition of oil is required to maintain products of high quality. It is of high importance that the oil is easy to handle. The main advantages of replacing palm oil with Swedish rapeseed oil are that rapeseed oil is better from a health perspective, as well as from an environmental point of view. Other advantages are that rapeseed oil is available in smaller quantities, it is cheaper and easy to handle. There is no need to heat the oil to make it liquid at room temperature, which will reduce costs and simplify the handling by enable elimination of heating equipment and electrical wires.

Before exchanging palm oil for rapeseed oil, Santa Maria AB is recommended to perform a full-scale production test for a longer period of time, using rapeseed oil. The samples of products of Italian Salad Seasoning and Taco Spice Mix, being manufactured with rapeseed oil in the present work, should also be evaluated after storage in elevated temperature and room temperature.

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