CHALMERS

Replace Synthetic Flavorings with Natural Flavorings

Product Development and Method for Accelerated Shelf Life Studies Development

Master of Science Thesis in the Master Degree Program Biotechnology

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CHALMERS UNIVERSITY OF TECHNOLOGY
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Cover:
The products worked with in the project; the Original cheese chips and Cheese dip mix in original packaging as well as a serving suggestion. Photographer: Josefin Klangefjäll

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Abstract
Flavorings are concentrated products consisting of the flavor molecule together with carriers. Flavorings are divided into two major groups, synthetic flavorings and natural flavorings. The consumers are more and more demanding products without synthetic flavorings since they do not want to have "chemicals" in the food. This project was part of the replacement process at the food company Santa Maria AB. The aim was to replace the synthetic flavorings in the Tortilla cheese chips, and Cheese dip mix with natural flavorings or other natural components. Complementary to the product development work a method to do accelerate shelf life studies of seasoned tortilla chips was developed. From the study it was obtained that natural flavors are generally not as complete and clean in taste as the synthetic ones, but together with other taste boosting components it is possible to achieve the same or better taste profile as previously without a too high cost. Also an accelerated shelf life method based on high temperature stress, possible to use when doing product development work of seasoned tortilla chips, was developed. The shelf life of the Tortilla cheese chips with the synthetic cheese flavors replaced was measured with the method to be seven months.

Keywords: Natural flavorings, Flavorings, Accelerated shelf life study, Tortilla cheese chips, Cheese dip mix, Snacks

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Josefin Klangefjäll, Gothenburg, May 2015

Table of Contents

1 Introduction

Santa Maria AB started as a small spice shop in 1911 and since then the company has grown to be the biggest flavor company in north Europe. Today the company is part of the Finish food cooperation Paulig group. The company has a strategy to produce flavorsome concepts that are easy to use in the consumers own home. The products are divided into five different groups, Tex Mex, Spices, Asia, BBQ and India. (1)

When producing food products it can be necessary to add flavor components in a very concentrated form, such as flavorings. In some products, like snacks, it is not possible to get a rich taste without using flavorings and not compromise on texture or appearance. One example of this is cheese flavored chips. If one wants a crispy, thin chips with a lot of cheese taste it is necessary to add the molecules giving the cheese taste in a concentrated manner.

Since the consumers are more and more demanding products with natural sources (2), the food industry is striving to use natural (NA) flavorings instead of synthetic ones. To do this, the food industry needs to overcome several impediments with NA flavorings, such as unclean taste, high heat sensitivity, low pH sensitivity and high prices. To meet the consumers demand on natural foods without a too high price the food industry need to put in a lot of work to find good natural tasting components.

One thing the product developers need to examine when developing a new, or changing an existing, product is the shelf life of the product. For food products with several months or even years shelf life it is necessary to have accelerated shelf life methods to use. One way to do accelerate shelf life studies is to expose the product to a high temperature.

Santa Maria AB aims to replace all synthetic flavorings with NA flavorings or other natural components before the year 2020, according to their product strategy.

1.1 Objectives

The purpose of this Master's Thesis is to evaluate if it is possible to replace the synthetic flavorings in the two Santa Maria products Tortilla cheese chips and Cheese dip mix without compromising taste, price or quality. The aim was that the new product should score same or higher in internal sensory panels and have an increase production cost of maximum 10 %.

To evaluate the quality over time of the new Tortilla cheese chips a method to measure the shelf life should be developed.

1.2 Limitations

Due to limited time the quality over time of the new Tortilla cheese chips was examined by an accelerated shelf life study and not in real time. The shelf life was evaluated when all cheese flavorings had been replaced, not for the final product. This is due to limited time. And also due to limited time the quality over time of the new Cheese dip mix was not evaluated.

2 Theory

To get a food product to taste just as you want, you can add tasty food components, spices or flavorings. Flavorings are mainly used where it is not possible to add a food component or spice to get the taste. Flavorings are, in most cases, more stable and easier to handle than comparable food sources and these are important properties for components used in industrial production of food, so in industry sometimes it is required to use flavorings to get the right taste. (3)

2.1 Definition of flavorings

The word ‘Flavorings’ is an umbrella term for different types of molecules, divided into five categories; flavoring substances, flavoring preparations, thermal process flavorings, smoke flavorings and other flavorings, according to the European flavor association (3). Below is a description of these categories.

Flavoring substances are chemically defined substances with flavoring properties (3). A chemically defined substance is a substance with constant composition (4). Today around 10 000 different flavoring substances have been identified in nature, and the flavoring industry uses around 2 500 of these. Depending on the manufacturing process flavorings can be divided into two major groups; natural flavoring substances and synthetically produced flavoring substances. The production of natural flavoring substances will be described in detail later. For the synthetic ones, two subcategories exists; nature-identical and artificial flavoring substances. Both of these are produced by chemical methods. As the names denote, the nature-identical flavoring molecules can be found in nature whilst the artificial ones cannot. (3)

Flavoring preparations are not chemically defined substances but a mixture that is defined by its raw materials. Examples are vegetable extract, fruit extract, spice and herb extract and yeast extract. Extract is defined as a product extracted and concentrated from a raw material (5).

Thermal process flavorings are flavors produced industrially by the controlled heating of raw material. For example, the flavors produced by the Maillard reaction, as in the case of baking bread or roasting meat.

Smoke flavorings are obtained by burning hardwood and collecting the smoke. The smoke is condense and mixed with a solvent or carrier. (3) Smoke flavoring have to be declared as “Smoke flavor” or “smoke flavor from” followed by the food, food category or source of the flavoring, such as oak. (6)

2.2 Production techniques of natural flavorings

Natural flavoring substances (NA flavorings) have a vegetable, animal or microbial origin. Primarily, a vegetable or animal source is used, but in cases were the production is too complex, the source material is not available in large enough quantities or the concentration of the desired compound is too low in the natural source (7), the production can be made by biotechnological techniques. The techniques used are plant cell cultures, microbial cultures and enzyme-catalyzed reactions. (3)

2.2.1 Plant cell culture

A culture consisting of plant cells is simply the definition of a plant cell culture. Plant cell culture is simply a culture consisting of plant cells. These cells are homologous with the cells in the original plant but are cultivated as single cells. Plant cell cultures can be used for production of low molecular weight molecules (8) and have many advantages over production in whole plants. These advantages are for example that cell cultures are without contamination of agrichemicals and fertilizers, it is not affected by pests, is independent of geographical and seasonal variations and production is faster (9-11)

Plant cell cultures can be used to produce flavorings. In some cases the flavorings produced differ from the one produced in the original plant (11), in this way new natural, non-GMO, flavorings can be obtained. This is a promising technique for production of NA flavorings which does not require large land areas, use of fertilizers, or other agrichemicals.

2.2.2 Microbial culture

One way to produce flavorings, is to use microorganisms. They can be used *in situ* as part of the food production for flavoring or they can produce the flavorings that then will be extracted. (11) The production is done in conditions that favor the production of the flavoring. It is possible to customize an organism producing the flavoring under suitable conditions, but the food industry in EU has banned GMO-produced flavorings so it is important to choose the transformation technique with care.

2.2.3 Enzyme-catalyzed reactions

The formation of flavorings in different systems can be catalyzed by the addition of enzymes. The enzymes are produced by microorganisms. (3) For example can the production of flavors in cheese be sped up by two to four folds by the addition of proteases and lipases. (12)

After the production, whether it is in a vegetable, animal or a microorganism, the flavor has to be isolated. This can be done either through extraction or distillation. Extraction can be done by several different techniques but the most commonly used are pressing or using a solvent. The distillation process is based on the fact that different substances have different boiling point. For example, if one wants to separate the flavor from the lemon oil you can heat the solution and then collect the steam at the temperature corresponding to the boiling point of the flavor. (3)

2.2.4 Problems with NA flavorings

It is a challenge for the food industry to change from synthetic flavorings to NA flavorings since the natural ones are coupled with some problems. First of all, many NA flavorings are less stable, more sensitive to heat and more sensitive to low pH than the synthetic ones. Also the quality of the NA flavorings is more dependent of seasoning variations. The last thing, worth mentioning, is that the price of NA flavorings is generally much higher than for the synthetic ones. (13)

2.2 Labelling rules

Depending on the composition of the NA flavorings in a food product different labelling can be used. If a flavoring originates from a source it can be connected to, and the taste can be recognized by the consumer, the labelling should contain one of the following; the name of the food, food category, vegetable or animal source. (14, 15) One example of this is if a pear flavoring is partially or completely obtained from the pear fruit it should be labelled together with ‘pear flavoring’. How the full labelling should look like is dependent on how big part the flavoring that is obtained from pear. This is divided into two parts:

- If >95 % by weight of the flavoring is obtained from the source material referred to: **Natural X flavoring.** Where **X** is one of the following: food, food category or a vegetable or animal flavoring source (14, 15) (in the pear flavoring case it would be: Natural pear flavoring)
- If <95 % by weight is obtained from the source material referred to: **Natural X flavoring with other natural flavorings.** Where **X** is one of the following: food, food category or a vegetable or animal flavoring source (14, 15) (in the pear flavoring case it would be: Natural pear flavoring with other natural flavorings)

The labelling **‘Natural flavoring’** without refereeing to any source may be used if a reference to the source material would not reflect the taste, if the taste of the flavoring is hard to recognize or if the flavoring is derived from different sources. One example could be a banana flavoring consisting of a combination of NA flavorings from strawberry, apple and raspberry. The recognized flavor is banana, but the added flavorings do not come from banana so in this case the label would be **‘Natural flavorings’.** (14, 15)

2.3 Cheese flavors

Cheese is a fermented milk product that is made by the addition of a starter culture and enzymes to a sample of milk. The starter culture has several functions; it is producing lactic acid, metabolizes citric acid, breaks down proteins, contributes to the breakdown of triglycerides and breaks down hippuric acid to benzoic acid. The produced lactic acid has in turn many roles in the ensuing cheese production. It causes, together with the rennet, the curd to form, it acts as a preservative and it contributes to the acidic flavor of cheese. The metabolism of citric acid is regarded as essential for the flavor development in cheese. Different starter cultures are used for different kinds of cheese, but all freshly-made curds are bland and have similar taste regardless of which starter culture was used. (16)

After the formation of the curd, it is ripened. During which the diverse flavors are produced. If a cheese is eaten immediately after manufacturing, before the ripening process, the flavor is the result of the action of the starter bacteria and is mainly due to diacetyl and possibly acetylaldehyde. In contrast, the flavor of a matured cheese is due to the interaction of starter bacteria, enzymes and the secondary flora (the starter culture is the first flora and the secondary is other microorganisms in the cheese, such as for example mold). The starter bacteria contribute to the flavor of matured cheese in different ways, one of these is the release of enzyme when the bacteria die. (16)

The ripening process can be for different amount of time depending on that the duration of the ripening process is adjusted to the cheese composition, the process temperature and activity of the microorganisms and enzymes present. During the ripening different biochemical reactions occur, the most important ones are (17):

- The metabolism of residual lactose, lactate and citrate
- Release of free fatty acids (FFA) (lipolysis), and associated catabolic reactions
- Degradation of the casein matrix into peptides and free amino acids (FAA) (proteolysis), and preceding reactions involved in the catabolism of FAA

During the above biochemical pathways several different compounds are produced which contributes to the flavor of cheese. Several hundreds of different compounds have been identified as part of the flavor of cheeses. (17) Some of these only exist in a specific type of cheese and some are in all cheeses. One example of a reaction that contributes to a flavor all cheeses have is the breakdown of proteins. This reaction contributes to the background savory all cheeses have, and this is the so called 'cheese flavor'. (16)

One of the companies supplying flavorings for this project makes natural cheese flavorings by analyzing cheeses to find out what molecules are contributing to the taste, see table 1 for some of the compounds contributing to Cheddar cheese taste. Then the company tries to find these molecules in other, cheaper sources and from these they extract the flavoring molecules. This flavoring needs some carriers, for example maltodextrin, and other compounds to work in a proper way. The flavorings used in this project are produced by making small drops of fat mixed with the flavoring compound(s) and have this drop covered with maltodextrin. (18) The maltodextrin, small oligosaccharides of glucose derived from starch (19), is used as a carrier of the flavor.

Table 1. Some of the flavor compounds in Cheddar cheese (17)

acealdehyde	n-propyl butyrate	n-pentanoic acid	ethyl butyrate
2-undecanone	propenal	2, 4-pentanediol	n-hexanal
3-methylbutyric acid	propanal	2-octanol	n-hexanol
2-tridecanone	n-propanol	n-octanoic acid	2-heptanone
thiophen-2-aldehyde	pentan-2-one	d-octalactone	2-butanol
tetrahydrofuran	2-pentanol	2-nonanone	acetoin
3-methyl-2-butanone	2-methylbutanol	methional	Acetone
3-methylbutanol	methyl acetate	methane thiol	Acetophenone
Isohexanal	n-hexanoic acid	d-dodecalactone	diethyl ether
Isobutanol	ethyl hexanoate	dimethyl sulphide	dimethyl disulphide
2-hexenal	2-ethyl butanol	Ethanol	n-butyl butyrate
2-hexanone	ethyl acetate	Diacetyl	n-butyric acid
hexanethiol	d-decalactone	dimethyl trisulphide	n-butyl acetate
Butanone	b-angelicalatone	1, 2-butanediol	n-butanol
p-cresol	n-decanoic acid	g-decalactone	carbon dioxide

Another way to get food product to taste like cheese without using ordinary cheese, is to add cheese-powders. Cheese-powders are made by adding water and melt salt to the cheese, this procedure makes the cheese melt, which is later dried, and a powder rich in cheese-flavor is obtained. (20) In this project different types of NA flavorings and cheese-powders will be used.

2.4 How tortilla chips are manufactured in the factory

The production of tortilla chips at Santa Maria AB is a continuous process. It starts with the mixing of the dough consisting of corn flour, water and salt. This mixture is then kneaded in a mixing device to make it elastic. The kneading time is dependent of the flour quality and may vary between 4 to 10 minutes. When the dough is ready it is sheeted in two steps to get it thin. The sheet of dough is then cut into the typical triangle shape that tortilla chips have. These chips are then baked and fried. The oven used is an IR-oven consisting of three different levels with different temperature range. The purpose of the baking step is to make the water content lower in the chips and to enhance the flavor. (21) Chips with lower water content will take up less oil during the deep-fat frying step (22).

When the chips are baked they have a dry surface and a moist inside. If they would be deep-fried right away they would pop up forming a cushion with air inside. This is unwanted in the production of tortilla chips hence before the frying the moisture needs to be even out. Since the water spontaneously will spread through the chips with time they are simply transported through the ambient air on a conveyor. After a specific, in beforehand set time, the chips are brought to the fryer filled with hot sunflower/rapeseed oil mixture. The product is fried for a few minutes. Now the chips are ready for seasoning. (21)

2.2.5 Seasoning of tortilla chips

After the chips are fried they are transported to the seasoning station. When they arrive they have a temperature of 40-60^{O}C (23). The warm temperature will melt the oil in the seasoning and make the seasoning stick to the chips. The amount of chips coming to the seasoning is constantly measured and the correct amount of seasoning is then added. The seasoning and the chips are continuously blended in a rotating drum. Now the product is ready to be packed. This is done by weighing up the right amount of chips, drop them into a bag and then sealing the bag. (21)

2.5 Packaging material

The majority of the food products on the market are packed in some kind of packaging material. This is done due to several different reasons; to prolong the shelf-life, to prevent contamination and leakage, to protect the food from hazards and to provide a convenient way of handling the product. For the preservation of the product and prolongation of the shelf-life, the packaging material is an important factor. The material should decrease the deterioration of both biotic and abiotic spoilage. Biotic spoilage is spoilage due to organisms and abiotic spoilage is defined as physical or chemical changes due to factors such as moisture, temperature, light and oxygen. In the case of tortilla chips it is mainly abiotic spoilage that reduces the shelf-life, and hence what the packaging material must be resistant to. Biotic spoilage is normally not a problem in products with a moisture level under 65 % (chips have approximately under 3 %). (24)

Chips are dry products that have a high fat content. For the texture it is crucial to prevent the product from absorbing moisture. When it comes to the flavor it is important to prevent the fat from oxidizing and causing rancidity. The oxidizing process is complex but the most important factors affecting it are availability of oxygen and exposure to light, the rancidity process will be described in more detail later. To prevent the chips from fat oxidation and to maintain the texture the chips need to be stored in a packaging material providing a barrier against moisture, oxygen and light. A metallized film is usually used to prevent the light from penetrating the product. (24)

The packaging used for the tortilla chips at Santa Maria AB consists of oriented polypropylene (OPP) laminated against a metallized OPP (25). In simpler words a plastic bag with a metallized coating. Plastic bags are categorized as partial barriers. Total barriers are such as glass and metal thicker than 1.7 µm. The OPP prevents moisture and oxygen from penetrating the product and the metallized layer prevents the light from reaching the product. (24)

2.6 Shelf-life

In this part the shelf life of food products in general and tortilla chips in specific will be discussed and a method to measure the shelf life will be presented.

2.6.1 Storage of food

The shelf-life of a food is the time it takes until the product becomes unacceptable for consumption. It can be in a safety, nutritional or sensory perspective. For short-life products it is mainly microbiological changes that are of primarily importance, while it is chemical and sensory changes that is the reason for degradation of medium- to long-life products (26). These different changes for degradation are influenced by different factors. The factors can be divided into two groups; intrinsic and extrinsic factors. (27) The intrinsic factors are the properties of the final product, and extrinsic factors are such factors that the product encounter during the food chain (production, distribution and storage). Some examples of intrinsic factors are:

- pH value
- Water activity
- Available oxygen
- Nutrients
- Natural microflora
- Raw material (e.g. use of preservatives)
- Natural biochemistry (e.g. enzymes)

Examples of extrinsic factors, if not specified the factor is influencing during the whole food chain:

- Relative humidity
- Exposure to light
- Environmental microbial counts
- Consumer handling
- Time-temperature profile during production
- Temperature control during storage

During the production of tortilla chips most of the water in the product is replaced with oil, hence the water activity is low. Also the product is heated to a very high temperature during both the baking and frying step so almost all microorganisms are killed. After this they are transported in the ambient air until they are packed into bags that immediate are sealed. It is during a few minutes the chips are cold enough for microorganism to survive on them until the bags are sealed, hence it is a very limited number of microorganisms in each bags. Due the low water activity these microorganisms are not part of the spoilage of the chips, if the sealing not is broken. So for the tortilla chips it is mainly chemical changes that occur during storage.

2.6.2 Rancidity

One of the most common chemical processes during storage of products containing fat is rancidity (28). Rancidity processes are divided into two subcategories; oxidative rancidity (caused by fat oxidation) and hydrolytic rancidity (caused by fat hydrolysis) (29). Rancidity causes off flavor and odor of the product.

The tortilla chips have high fat content so in these the main process that determines the shelf-life is the rancidity process. The spice mixture contains a small amount of oil, it is possible that this will cause rancid off flavor and odor during the storage but also other components in the mixture may undergo chemical changes. Common deterioration mechanisms in spices and spice mixtures are microbial growth, volatile loss, chemical reactions and moisture uptake (28). These mechanisms lead to mold or bacteria growth, flavor changes, color loss and caking.

The shelf-life of a product may be calculated from standard values, measured in real time or by accelerated shelf-life tests. In both real-time and accelerated shelf-life tests the product is evaluated by sensory panels or/and by instrumental methods. Sensory panels are essential to evaluate perceived quality of the product, but the method is expensive, time consuming and suffers from high variability. If valid instrumental methods are available they may be used as replacement or complement to the sensory evaluation. (28) In this project the shelf-life is evaluated by accelerated shelf-life and analyzed by a trained sensory panel.

2.6.3 Accelerated shelf-life test

On today's food market a lot of new products are launged each year. The companies behind these products want to be first on the market with a new concept, and the competitive companies want to produce a copy fast to be able to take a piece of the profit. This desire of fast development has had a hold up point, the shelf-life testing. A food product can have several years of shelf-life, and the companies do not want to wait that long to be able to get a valid shelf-life test. To solve this problem, researchers have developed several different techniques to do accelerated shelf life test. The common thing among these tests is that the product is tested during conditions that accelerate the process making the product get bad. (30)

To make an accelerated shelf-life test the reaction rates of the deteriorating mechanisms need to be speeded up. This can for example be done by using higher temperature, higher water activity or different pH, all conditions compared to normal storage conditions. (30) Depending on the reaction supposed to deteriorating the food, the exposing condition is chosen.

To measure the shelf-life it is also important to distinguish a measurable quality parameter that is affected by the chosen exposure condition. This parameter can be physical, chemical, microbial or sensory indices. The change of this quality parameter (A) with time can be expressed as equation 1. It is also possible to measure the gain of an undesirable parameter (B). The rate of formation of B is expressed in equation 2.

$$\frac{-d[A]}{dt} = k[A]^n \qquad \text{(e.1)}$$

$$\frac{d[B]}{dt} = k' * [B]^{n'} \qquad \text{(e.2)}$$

In equation 1 and 2, k and k' are the reaction constants and n and n' are the apparent reaction orders. For constant temperature it is possible to solve the rate equation into a linear expression dependent on time (t):

$$f(A) = k * t \qquad \text{(e.3)}$$

where f(A) is the quality function of the food. (31)

The quality loss of food products normally follows zero orders or first orders kinetics. For the loss of the quality parameter A the equation 1 would turn into equation 4 for a zero order reaction, and equation 5 for a first order reaction.

$$[A] = [A_0] - k * t \qquad \text{(e.4)}$$

$$\ln([A]) = \ln([A_0]) - k * t \qquad \text{(e.5)}$$

To evaluate if the reaction is of zero or first order, [A] should be plotted against t as well as ln([A]) against t and the correlation coefficient of the two plot should be compared. R^2-value close to one means a good fit.

2.6.3.1 Arrhenius model

To compare the accelerated shelf-life result to real time shelf-life, different methods may be use. When using temperature as accelerating condition, the Arrhenius model is recommended to use. In this model the temperature effect is incorporated into an exponential model of the rate, equation (6).

$$k = k_a * e^{\frac{-E_a}{R*T}} \qquad \text{(e.6)}$$

In the equation k_a is the pre-exponential factor, E_a the activation energy (cal/mole), R the gas constant (R = 1.987 cal/(K*mole)) and T is the temperature (K). The activation energy is a degree of the temperature sensitivity of the reaction. If the activation energy is between 10-20 kcal/mole it is an indication of a simple hydrolysis reaction, if it is 15-25 kcal/mole it may be lipid oxidation by free radicals, enzymatic browning normally have an activation energy of 20-40 kcal/mole and enzyme or microbial destruction 50-150 kcal/mole. (32)

2.7 Sensory analysis

Sensory analysis is a discipline used to evaluate the reaction a food or other material have on the senses (sight, taste, smell, touch and hearing). The analysis can be performed both with humans as instrument and with hardware such as an electronic nose. The most used technique today is with a human panel as evaluator. This panel may consist of trained panelists or of consumers depending on what kind of test is desired. (33)

In the food industry sensory analysis is used when a product is developed or changed. It can for example be to make a product more desirable for the consumer, make it cheaper without changing the taste or to develop a new product. It is important to perform the test in such a way that different impacts are avoided. Preferable the test is performed in a room where the attendants can evaluate the food without being disturbed by different noises, smells or by the other panelists. This room should have neutral colors, one booth for each panelist and with the possibility to change the light in a way that the color of the food can be masked. (34, 35)

Other things that may affect the panelists are the code for each sample, the order of the samples and the color of the serving materials. The panelists may be affected if the samples are named for example A, B and C or 1, 2 and 3, some people connect 1 and A to ‘the first’ or 3 to ‘the highest’. Such associations will affect the results from the analysis. Studies have shown that if a three number code is used this effect may be considered as insignificant. (35) The order of the samples affect since the attendant will compare the sample with the previous tested, hence all possible serving orders should be used equally many times during a sensory analysis. Also the color of the plate, bowl or cup, used to serve the food, affect the consumer. Preferable is neutral and white serving material. It is also important to make sure that the attendant can neutralize the mouth between each sample. This can be done by rinse the mouth with water or eating some neutral rice or crackers. (35)

As mentioned earlier, the panel can consist of trained panelists or of consumers. Consumers are used if it is meant to evaluate if a difference of a product have any value for the consumers and an trained panel to get more information about how the difference effect the product. (34) In this project both an internal consumer panel and an internal trained panel will be used. Below is a presentation of the different sensory analysis method used in this project.

2.7.1 Triangle test

The triangle test is used to evaluate if there is a detectible difference between two products for consumers. The products, A and B, are served three at a time and the tester is supposed to pick out the one that differs from the others. It is important to use all possible test orders and a large enough panel. The panel may consist of either trained panelists or of consumers. It is a 'forces choice' test, meaning that if the tester cannot notice a difference between the samples, he should guess. (36)

To evaluate the results, the number of right answering panelist is compared to tabulated values, depending on significance level and total number of panelists in the test.

2.7.2 Paired preference test

A paired preference test is performed to evaluate which of two products a consumer prefers. The two samples are served two at a time, and the two possible serving orders should be used equal many times. The panelists are asked to mark the sample they prefer. The test is a two sided test, hence both samples can be preferred, and should be evaluated as one. In the evaluation all answers are summarized and categorized depending on which sample the panelist chose. The largest summarized value is compared to tabulated values, dependent on significance level and total number of panelists in the test. (35) (37)

2.7.3 Descriptive test

Descriptive test is a collection name for different methods, including Flavour Profile Method (38), Texture Profile Method (39), Quantitative Descriptive Analysis^{TM} (40), Spectrum^{TM} method (41). For all of them a trained panel is needed and the methods are normally used when it is known that it is a detectible difference between two or more products to evaluate what and how big that difference is. (35, 36)

All methods are divided into three parts; definition of the attributes that should be evaluated, training of the panel on the attribute and evaluation of the intensity of the chosen attribute on a scale from low to high intense for each product and each attribute. The difference between the methods is how these three steps are executed. (35, 36)

In this project the Consensus sensory profile was used for the shelf-life studies. In this method a group of four to ten trained panelists are evaluating products together by consensus. The panel develops its own terminology and scores pertaining to the sample set presented. During the analysis the panel should be seated around a table on which reference samples may be available. Advantages with this method are that more samples (up to ten similar items) may be evaluated at the same occasion and the training time of the panel may be reduced. A disadvantage is that the quality of the data is very dependent in the experience of the panel leader. (42)

2.7.4 Quality test

This method is used to check the quality of a product. In this method the panelists give a point to each product for each attribute evaluated. The scale used is normally the 9-point hedonic scale which goes from "dislike extremely" to "like extremely" (43). It is possible to use a scale with fewer steps (for example 5-points). This can be good when the panelists are not capable to evaluate on a 9-point scale. Children are an example of panelists that can find it hard to evaluate on a 9-point scale, for them it is good to use few steps and possibly symbols, such as different levels of smiling faces. (34) The 9-point scale has other limitations, like people tend to center their judgment around the center values and avoid the end-points (44), but overall it is a good method to get an indication of general levels of preference (43).

The quality test is often used to evaluate if a current product meet the specification. If it is evaluated on the 9-point hedonic scale, the product has to be changed if it scores six or lower, and not reach the consumers if it scores tree or lower, according to Norsk institutt for nearingsmiddel (1985) (36). It is also possible to use this method to evaluate if a product scores higher than another, for example two different brands or a new recipe compared to an old. (36) To evaluate if a difference exist a *t* test, described below, may be performed on the data.

2.8 *t* test

t test is a statistical model used to compare two normally distributed sample populations, X and Y. The method is based on two hypothesis, the null hypothesis, H_0, and the alternative hypothesis, H_A. The following hypotheses are used: $H_0: \mu_X = \mu_{2Y}$ (no difference in the true average value of the considered characteristic of the two samples) and $H_A: \mu_X \neq \mu_Y,\ \mu_X < \mu_Y\ or\ \mu_X > \mu_Y$ (difference in the true average value of the considered characteristic of the two populations). In a *t* test the conclusion is if the null hypothesis can be rejected or not. By the test two errors may be done; α: rejecting H_0 even thou it is true, and β not rejecting H_0 even thou it is false. When doing the calculations one choses how big risk of doing an α error that is acceptable, this is called the significance level, usually a 5 % significance level is used (α = 0.05). (45)

When the alternative hypothesis $H_A: \mu_X \neq \mu_Y$ is used, a so called two-tailed *t* test is performed (45). This method was used to evaluate the data from the sensory analysis in this study and is described below.

A test statistic t_{obs} is calculated by using equation 7 where $\bar{x}$ denotes the average value, s_1 the sample variance and n_A the test population of the first sample population, A. $\bar{y}$, s_2 and n_B represent the same values but for second sample population, B. (45)

$$t_{obs} = \frac{\bar{x}-\bar{y}}{\sqrt{\frac{s_1^2}{n_A}+\frac{s_2^2}{n_B}}} \qquad \text{(e.7)}$$

The sample variance is calculated with equation 8, where x_i denotes the individual values of the respective sample population, with test population n. (45)

$$s^2 = \frac{\sum(x_i-\bar{x})^2}{n-1} \qquad \text{(e.8)}$$

Degrees of freedom are calculated with equation 9 (45), ν should be rounded down to nearest integer.

$$\nu = \frac{\left(\frac{s_1^2}{n_A}+\frac{s_2^2}{n_B}\right)^2}{\frac{\left(s_1^2/n_A\right)^2}{n_A-1}+\frac{\left(s_2^2/n_B\right)^2}{n_B-1}} \qquad \text{(e.9)}$$

The test statistic t_{obs} is compared to tabulated t-value to evaluate if there is a significant difference between the sample populations or not. To obtain this tabulated t-value, the degrees of freedom and the significance level is necessary to know. When a two-tailed *t* test is performed the $t_{\alpha/2,\nu}$ is read. H_0 is rejected if $t_{obs} \leq -t_{\alpha/2,\nu}$ or $t_{obs} \geq t_{\alpha/2,\nu}$, which means that with a significance level α, the two sample populations have different true average values of the considered characteristic. (45)

3 Method

The project's focus was to replace the synthetic flavorings with NA flavorings or other natural components in the Tortilla cheese chips and the Cheese dip mix. An accelerated shelf-life test method for Tortilla cheese chips was also developed.

3.1 Product development – Tortilla cheese chips

In this part the replacement process of flavorings in the Tortilla cheese chips is described. Firstly a description of how seasoned chips were manufactured, than the actual replacement process.

3.1.1 How chips are manufactured in the test kitchen

The seasoning for the chips was developed, see description below, in the test kitchen at Santa Maria AB. To test it in its application the seasoning mix was applied on unseasoned chips obtained from Santa Marias's taco factory in Mölndal, Sweden. The chips where picked out from the production line after the frying step, and stored in a plastic bag until use, the chips where refreshed with three to six weeks interval to make sure of fresh samples. In the test kitchen they were heated up in an oven until the surface temperature was 75°C - 90°C, measured with a Fluke 65 infrared thermometer. With a temperature of 75°C - 90°C when the chips leave the oven, the chips are 40-60°C when the seasoning is added, the same temperature as in the production. After the heating step the chips are added to a plastic bag together with the spice mixture (6.54 % by weight spice mixture). The bag is sealed with a Fermant 40 N sealer and shaken for a minute to distribute the seasoning on the chips.

3.1.2 Replacement of synthetic flavorings in the Tortilla cheese chips

The Tortilla cheese chips produced today contain four cheese flavorings (Cheddar, Emmental, Oaxaca and a Cheese booster flavoring) and one jalapeño flavoring, called Jalapeño-α in this report. The Cheese booster flavoring consists of mainly salt and yeast extract as well as some flavoring components. The first step in the replacement process was to find out what the distributor of the flavorings could offer when it comes to NA flavorings. They were asked to match the flavoring used today. To do this matching the distributor needed to know the pH of the spice mixture. This was analyzed by the laboratory at Santa Maria AB with three replicates, the results can be found in Appendix I.

For the Cheese booster flavoring no natural equality was obtained. For the cheddar-flavoring there were three alternatives, these are called Cheddar-1, Cheddar-2 and Cheddar-3 in this report. For the Emmental flavoring and the Oaxaca flavoring one alternative to each was obtained, called Emmental-1 and Oaxaca-1. For the Jalapeño-α flavoring, two alternatives were obtained, Jalapeño-1 and Jalapeño-2. To do the replacement, sensory taste profiles of all flavorings was needed. This was analyzed by two to four R&D specialists at Santa Maria AB with the flavorings dissolved in water (0.05 - 0.15 % by weight). The R&D specialists are verified by SP Food and bioscience to be able to sense different tastes in a professionally way.

<u>The artificial flavorings used today:</u>

Cheddar flavoring: Rich taste of hard, matured cheese, sweet

Emmental flavoring: Nutty taste, creamy, cheese sweetness

Oaxaca flavoring: Cream, butter, intense

Cheese booster flavoring: Salty, creamy, yeast extract

Jalapeño-α flavoring: Heat

The obtained natural alternatives:

Cheddar-1: No real cheese flavor, detergent taste, artificial taste

Cheddar-2: Cheese taste, “Prästost”, metallic aftertaste

Cheddar-3: Creamy taste, Good cheese that have gone bad, ‘Blue cheese’, strange off-taste

Emmental-1: Old cheese rind, artificial taste

Oaxaca-1: Fat, mineral taste, coconut, tropical fruit

Jalapeño-1: Green-vegetable, bell pepper

Jalapeño-2: Hot, vegetable

For the Cheese booster flavoring, different replacement alternatives were evaluated. Firstly the different alternatives were tasted in hot water (0.1 % by weight), and compared against the Cheese booster flavoring in hot water (0.1 % by weight), see table 6 (in section 4.1) for all mixtures tested. The best alternative was tested in chips. From the three cheddar flavorings, Cheddar-1 and Cheddar-2 was selected for further analyze, since they match the original cheddar flavoring the best out of the three alternatives. To find good concentrations to use of the two Cheddar flavorings as well as Emmeltal-1 and Oaxaca-1, they were tested in chips one by one, see table 7 (in section 4.1) for all concentrations testes.

Cheddar-2 was selected over Cheddar-1 because of a lower price tag and better taste (according to five R&D specialists at Santa Maria AB).

The Oaxaca-1 did not give a good taste in the chips, hence other alternatives were evaluated. Since the taste profile of the synthetic Oaxaca flavoring was mostly butter taste, two natural butter flavorings were tested as replacements. Profile of these tasted in water (0.1 % by weight) by two R&D specialists at Santa Maria AB:

Butter-1: Butter

Butter-2: Popcorn-butter

These two butter flavorings were tested together with the other NA flavorings in chips, see table 8 for a description of the samples. With Butter-1, called Chips 14, an appropriate alternative to the original was obtained. To verify that the difference between this chips and the original was unnoticeable for consumers, a triangle test with the internal consumer panel was performed. All possible serving orders were used, for an example of test protocol used see Appendix II. To get rid of the taste between the different samples, wheat wafers and water were used. To verify if there was a difference between the two samples, the result was compared to a tabulated value using the significance level $\alpha = 0.05$.

The price of the Chips 14 was calculated. Since it was slightly higher compared to the original it was desirable to make it cheaper. One suggested way was to add different cheese powders and lower the amount of flavorings. Three different vegetarian cheese powders were evaluated, Powder-1 – 3. They were tested in different amount in the spice mixture, together with the natural cheese flavorings. See table 8 (in section 4.1) for the different chips tested.

With Powder-3 instead of the original cheese powder (Chips 23), the same taste profile was obtained and the spice mixture was 1.31 % cheaper than Chips 14, which has the original cheese powder.

The last thing to do in the development work of the chips was to replace the last synthetic flavoring, the Jalapeño flavoring. It was tested to be replaced with Jalapeño-1 and with chili powder, table 8 (in section 4.1) for the different mixtures tested. By replacing the flavoring with only chili powder (0.67:1) a chips, close in taste to the original, but with no synthetic flavorings, was obtained, called Chips 27.

A panel consisting of four R&D specialists noticed an unpleasant smell from freshly made Chips 14. This smell was originated from the NA flavoring Emmental-1. The product developer noticed that the smell evanesce a few days after production. To make sure that the aroma from the chips was pleasant when it arrived to the customers a hedonic evaluation of the aroma in newly opened bags was executed with the internal costumer panel. Small bags, with 50 ± 0.5 g chips were stored in room temperature for three days (the minimum time from production to customer). At the production time the latest chips produced was Chips 23, hence it was this chips, as well as the Original that was produced three days prior analysis.

The chips with no synthetic flavor, Chips 27, was developed one day before the hedonic evaluation. Since it was desirable to evaluate the taste of this chips, this was produced and evaluated in the hedonic evaluation the day after, together with the Chips 23 and the Original chips. During the analysis, each panelist was served three bags with chips, one of each type, all possible serving orders used, see Appendix III for an example to test protocol used. Both aroma when opening bag, taste and overall acceptability was evaluated on the hedonic scale for each sample.

The data was tested with the MATLAB function Normplot to verify normal distributed data. Then *t* test was done to evaluate if the samples significantly differed in the results. For calculations and MATLAB code, see Appendix III.

3.2 Product development – Cheese dip mix

In this part the replacement process of flavorings in the Cheese dip mix is described.

3.2.1 Replacement of synthetic flavorings in the Cheese dip mix

In the current cheese dip mix there is four synthetic flavorings and one NA flavoring. The synthetic ones are three different types of cheese flavorings and one jalapeño flavoring. The cheese flavorings are the same as in the cheese tortilla chips, the Cheddar, the Emmental and the Cheese booster flavoring. Whilst the jalapeño flavoring is another one called Jalapeño-β. The NA flavoring is an umami giving flavor rich in yeast extract, in this report the flavoring is called Yeast flavoring.

The distributer of the Jalapeño-β flavoring was asked for natural alternatives for it. The same alternatives as for Jalapeño-α was obtained. The natural alternatives had already been tested in hot water, see section 3.1.2, but the same needed to be done for the Jalapeño-β. Dosage 0.1 % by weight was used, and it was analyzed by three R&D specialists at Santa Maria AB:

Jalapeño-β flavoring: weak jalapeño taste, hot

First the cheese flavorings were replaced, then the cheese powder. In the original Cheese dip mix it was the same cheese powder as in the original Tortilla cheese chips, and it was desirable to keep having the same powder in both due to that the price is lower for bigger shipments of raw material. After this the jalapeño flavoring was replaced and two dips with no synthetic flavoring, and still a balanced, rich cheese taste, were produced. For all tested dips see table 11 in (section 4.2), all samples were prepared by mixing dip mixture with sour cream (8 % by weight dip mixture) and leave it in cold for 10 to 30 minutes. The dips were evaluated by two to four R&D specialists at Santa Maria AB. The two alternatives produced (Dip 13 and Dip 17) had a slightly different profile than the Original, they had a more full-flavored cheese taste instead of the sharp cheese taste the original have, according to three sensory specialists at Santa Maria AB. These two alternatives were tested together with the original Cheese dip mix on the internal consumer panel in a hedonic evaluation. Since one of the alternatives was clearly hotter in taste than the other two samples this was served last in the order to all participants. The other two possible serving orders were used equally many times, for an example of test protocol used, see Appendix IV. The panelists were served Tortilla chips and cucumber to taste the dip with, as well as spoons. To get rid of the taste between the different samples, wheat wafers and water were used.

Since only ten panelists participated in the evaluation it was decided to do a replication and in this test only test the best alternative out of the two, best according to the results from the first hedonic evaluation, Dip 17 together with the Original dip. At the same occasion a dip, called Dip 18, was tested that had the same proportion of NA cheese flavorings as the Tortilla cheese dip, since it is desirable to buy a pre-prepared mixture from the distributer due to a lower price tag. Except the proportion of the flavorings, Dip 18 is exactly like Dip 17. All six possible serving orders were used, see Appendix V for an example of test protocol used. The panelists were served Tortilla chips to taste the dip with, as well as spoons. To get rid of the taste between the different samples water were used. It should be mentioned that seasoning mixture for Dip 18 was mixed the same day as the evaluation. This is not optimum since flavorings need to stand minimum 24 h in the mixture to stabilize, according to R&D specialists at Santa Maria AB.

The data, from both hedonic evaluations, was tested with the MATLAB function Normplot to verify normal distributed data. Then *t* test was done to evaluate if the samples significantly differed in the results. For calculations, MATLAB code and graphs, see Appendix V.

3.3 Accelerated storage test of Tortilla cheese chips

A method to evaluate the shelf-life of Tortilla cheese chips was established, and the method established was used to evaluate the shelf-life of the developed Tortilla cheese chips when all synthetic cheese flavorings had been replaced, Chips 14. Due to limited time, no shelf-life study was executed on the final Tortilla cheese chips or on the Cheese dip mixture.

Two different methods were evaluated; the first one was rejected very fast due to poor results. Below both methods are described. For Method 1 only the seasoned chips were tested, and for Method 2 both the seasoned chips as well as only the spice mixture were evaluated. The reason for testing the spice mixture by itself was because it was anticipated that the rancidity of the chips would mask any changes in the seasoning. In both methods the temperature was used as exposure condition and three different temperatures were used for test samples. The method to evaluate the samples was also the same for both methods, sensory analysis with consensus profiling, this is described after the description of both methods.

3.3.1 Method 1

According to Taoukis and Labuza (1996) (46) dehydrated products, like chips and seasonings, should be tested in the temperature range 25-45°C with control samples at -18°C. According to Man et.al. (2002) (47), the procedure to use samples stored in a freezer as control samples works for potato chips. In this study the same method was evaluated for Tortilla cheese chips. The procedure was only done on the original chips to evaluate if it was a good method to use on Chips 14 as well. Chosen temperatures and test intervals can be found in table 2. Two climate cabinets of the same type (Gyllenkamp, INC-000-MA1.9/INC-104-488K/71100-907-1) were used for the 30°C and 42°C samples, and the room temperature samples (21°C) were stored in the same room to get the same humidity of the air for all samples. Room temperature was chosen as the third test temperature due to lack of space in available climate chambers. The temperature of both the climate cabinets and the room temperature was measured continuously during the analysis with a separate thermometer.

Table 2. Test temperature and sampling interval for Method 1.

Temperature	Test interval
21°C	Every two week
30°C	Every two week
42°C	Every week

The chips were stored in the same material as the chips are sold in today, a bag with oriented polypropylene (OPP) laminated against a metallized OPP. The bags are produced in two sizes, for 200 g and for 500 g chips. In this method 45 g ± 0.2 g chips was put in ¼ of a 500 g bag. The bags were sealed with a Fermant 40N sealer. As control samples bags of the same size with chips were put in freezer (-20°C). Since the panel not was ready to evaluate the 0-sample (at t = 0 days) when the test started, due to that the training of the panelists, see description in section 3.3.3.1, just had started, this sample was stored in the freezer (-20°C) for two and a half week until the panel was ready. The unseasoned chips were stored in one bag and the seasoning in one. When the panel was ready to evaluate the 0-sample, the chips were heated and spiced, as earlier described, the same day as the evaluation.

When a sample had been stored for the given time it was put in the freezer (-20°C), to stop any reactions happening in the chips, until evaluation. Also it was apprehended that the freezer might affect the chips in any way, and in that case it was necessary to have the same effect on both controls and test samples. After two and a half week the first samples were evaluated by the panel. The samples tested can be found in figure 11 together with the results.

3.3.2 Method 2

Since the freezing time seemed to affect the chips more than the heat time in Method 1, Method 2 did not have the freezing step. As control samples, freshly (made that day) seasoned chips were used each time, the spice mixture was maximum three weeks old. To control if different mixing occasions affected the results, two spice mixtures mixed at different occasions and four different chips, seasoned at different occasions, were evaluated by the panel blindly.

In this method, both the Chips 14 and the Original were tested to compare the shelf lives. Samples of both chips and spice mixtures were stored in three different temperatures. This time 30°C, 42°C and 50°C were used. The highest temperature, 50°C, is slightly above the recommendation from Taoukis and Labuza (1996) (47) this was used because the sample stored at 42°C in Method 1 did not show any big changes after two weeks of storage, and Method 2 was planned to be run for six weeks, so it was necessary to speed the reactions up additionally. According to the supplier of the flavorings, they would be stable enough to do the storage test at 50°C. The climate cabinets used were; Gyllenkamp, INC-000-MA1.9/INC-104-488K/71100-907-1 for both 30°C and 50°C, and Sanyo, MIR-152 for 42°C. All three cabinets were placed in the same room to obtain the same humidity of the air. The temperature of the climate cabinets was measured continuously during the analysis with a separate thermometer. The weight of every sample was measured before and after the storage.

For the chips, ¼ of a 500 g bag was used for each sample, filled with 50 ± 0.2 g chips. For the spice mixture, ¼ of the 200 g bag was used, filled with 23 ± 0.1 g. To analyze the effect of more air exposure of the spice mixture, a whole 200 g bag was used for one spice mixture sample (23 ± 0.1 g), called "Big bag" samples. This big bag was stored in 42°C and was analyzed at the end of the study. All bags were sealed with the same Fermant 40N sealer two to twenty hours before filling to get rid of the smell from the sealing, since the machine gives an uneven sealing smell in the bags it was desirable to get rid of it before filling.

For both the chips and spice mixture in 50°C, samples were taken every week for analysis. For the other two temperatures, samples were taken every second week for both the chips and spice mixture. The samples were kept in room temperature for a few hours to reach the same temperature before the analysis.

3.3.3 Sensory Analysis – accelerated shelf life study

For both Method 1 and Method 2 the samples were analyzed by an expert panel using the method Consensus sensory profiling. Since the panel leader lacked experience of sensory analysis in group before this study, it was required that the panel leader gained some experience before the analysis started. This was done because the quality of the data from Consensus sensory profiling is very dependent on the experience of the panel leader (42). How this experience was obtained is described in section 3.3.3.3.

3.3.3.1 Selection and Training of expert panel

The panel members were selected from the personal at Santa Maria AB. All R&D personal as well as the product manager for the tortilla cheese chips were asked if they wanted to participate. Five people answered that they wanted to participate, but since one of them missed out the whole training period that person was excluded from the expert panel. Of the remaining persons, three works at the R&D department and the last one is the product manager for the Tortilla cheese chips. To obtain a homogenous group, the panel was trained at three different occasions. At these occasions, descripted words for the taste of the chips and spice mixture were also selected. During the first meeting, a sensory expert joined the group to help the panel leader. For this occasion, all raw materials (except the coloring) in the spice mixture for Tortilla cheese chips were presented, as well as the NA flavorings obtained from the distributer. All flavorings were presented in hot water (0.1 % by weight) whilst the other raw materials were presented as dry matter. The chips and spice mixture of the Chips 1 were also served. Chips 1 was used as reference, instead of the Original since at the time the expert panel was assumed to be evaluating samples by using another method, since the Chips 1 and the Original were almost identical in taste (according to six R&D specialists at Santa Maria AB) it was decided to not be necessary to redo the training occasions with the Original as reference.

At the first training occasion the panelists were asked to taste all raw materials and to find the different tastes in the chips and spice mixture. During the tasting the group discussed from which raw materials the different tastes could come. The panelists were also asked to write down individually what words they would like to use to describe the taste of the chips and the spice mixture respectively, see Appendix VI for those words.

To make the panel have the same references for different cheese descriptive words the next occasion was dedicated to try different cheese types confounded with the flavorings in the chips. Seven different cheeses were presented, and the panelists were asked to try all of them and find out which matches the taste of the chips and spice mixture (Chips 1), Appendix VI for a presentation of the cheese variety and result of the tasting. Also the synthetic Cheddar and Emmental flavorings were presented in hot water (0.1 % by weight). The conclusion from this training occasion was that the cheese taste in the chips and spice mixture most closely tasted like cheddar cheese, but some of the panelists thought the cheese taste was more close to cheese doodles taste.

At the end of the second occasion the panelists were tested to see if they could notice what had been changed in three different spice mixtures and corresponding chips, one with extra sugar (50 % extra), one with extra salt (40 % extra) and one with extra Cheddar flavoring (30 % extra). They were served in white bowls coded with a three figure number. See Appendix VI for the results.

Since the panel still could not agree on how to describe the cheese taste the last training occasion was dedicated to try different cheese doodles, for a presentation of the cheese doodles, as well as results of the tasting, see Appendix VI. The panel did not think the Tortilla cheese chips or the spice mixture tasted like cheese doodles. During the tasting the panel was also served old chips to acquaint with rancid taste of chips. The chips they tried were old 'Heat spice and shake' chips, an earlier product in Santa Maria's selection, and they were 28 week over the expiring day. The panel noticed that they smelled more rancid than they tasted rancid. Since the rancidness was mainly noticeable in the odor, it was decided to analyze both smell and taste of all future samples. After this training occasion the panel was united with how to describe the taste and smell of both the Tortilla cheese chips and the corresponding spice mixture. These words, table 3, were used for Method-1.

Table 3. Words used to describe the taste and the smell of the chips and spice mixture in Method-1. Words in italic were used to describe the smell.

Chips	Spice mixture
Cheddar-cheese	Cheddar-cheese
Salt	Salt
Chili-heat	Chili-heat
Sweetness	Sweetness
Sourness	Sourness
Rancidness	Rancidness
Body	Body
Deep-fried corn	Paprika
Off-smell	*Off-smell*

Note: One panelist could note participate during the second training occasion, and another panelist could not participate during the last training occasions. For these two an individual training session was held.

3.3.3.2 Analysis

All analysis of samples was done by consensus profiling with a five point scale, previously developed at Santa Maria AB, table 4. The samples were served in bags marked with a three figure number, the storage samples in the same bag as they were stored in. The odor was analyzed by sniffing in the newly opened bag. To analyze the taste, one to four chips and two to five fingertips full of spice mixture were tasted by each panelist. For each sample the panelists were asked to do several tastings and to evaluate three to five specified attributes each time. After a tasting the panelists discussed and points were set for each attribute on the five grade scale. If the panelists were unsure what value to set, they were asked to go back to the reference (Original chips/seasoning mixture for Method-1 and Chips 14 and Original chips/seasoning mixture for Method-2) and compare it. To get rid of the taste between the samples, wheat wafers and water/sparkling water were used.

Table 4. Scale used for screeing at Santa Maria AB.

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

A screening profile of the original chips was necessary for both methods. This was evaluated by analyzing newly made chips and spice mixture with three replications to obtain consistent results. At the last replication, the panelists were told what they had said at the second analysis and were asked if they wanted to change anything. These values were then used as reference in the subsequent analysis. For the second method a screening profile for the Chips 14 chips and seasoning mixture was needed. This was analyzed by three replicates of the chips and two replicates of the seasoning mixture.

After the last analysis from Method-1 some new attributes were discovered in the chips and the spice mixture. These were added to the descriptive list used in Method-2, table 5.

Table 5 Words used to describe the taste and the smell of the chips and spice mixture in Method-2. Words in italic were used to describe the smell.

Chips	Spice mixture
Cheddar-cheese	Cheddar-cheese
Salt	Salt
Chili-heat	Chili-heat
Sweetness	Sweetness
Sourness	Sourness
Rancidness	Rancidness
Body	Body
Deep-fried corn	Paprika
Rancid smell	*Rancid smell*
Off-smell	*Burnt cauliflower smell*
	Off-smell

Note: All the panelists could not participate during all analyses.

3.3.3.3 Training of panel leader

The panel leader needed to get experience of both sensory analysis and panel leading. To get the first a pre study was executed with a so called "Save the raw material" project. An Emmental flavoring used in the 'Heat, spice and shake' product was going to be discarded due to production stop. It was desirable to save this flavoring by using it in the Tortilla cheese chips. The panel leader developed a recipe with this flavoring and did a paired preference test on the internal consumer panel. The two possible serving orders were used equally many times, for an example of test protocol used, as well as the result see Appendix VII. To get rid of the taste between the different samples, wheat wafers and water were used.

To get experience of how to evaluate and describe taste, the panel leader participated in the weekly sensory expert panel occasions at Santa Maria AB. During these occasions new raw material, new products or products from test runs are evaluated by three to five R&D specialists.

The last training step for the panel leader was to get experience of panel leading. For this the panel leader participated in a coconut milk expert panel. This panel evaluated different samples of coconut milk by consensus profiling.

3.4 Data handling

Most of the calculations were done with Microsoft Office Excel 2010, and a few calculations with MATLAB.

4 Results

The results from the study are presented in this part.

4.1 Product development – Tortilla cheese chips

For the chips several different mixtures, spice mixtures and chips were done. First every cheese flavoring was replaced one by one, see table 6-7 for a presentation of the mixture as well as the results from tastings by one to five R&D specialists at Santa Maria AB.

Table 6. Mixtures tested to replace the Cheese booster flavoring, and the chips tested with the best mixture

Sample	Description	Result
Mixture 1	Salt, light yeast extract and milk powder	A bit less full-flavored taste than the Cheese booster flavoring
Mixture 2	Salt, light yeast extract and Cheddar-1	Not close to Cheese booster flavoring
Mixture 3	As Mixture 1 but more salt and milkpowder	Better than Mixture-1
Mixture 4	As Mixture 1 but addition of lactic acid and cheese powder	Not close to Cheese booster flavoring
Mixture 5	As Mixture 3 but slightly less milk powder and light yeast extract, as well as addition of lactic acid	Close to Cheese booster flavoring
Chips 1	With Mixture-5 instead of the Cheese booster flavoring	Close to the original chips, four R&D specialists could not tell them apart when tasting blindly

Table 7. Different spice mixtures tested on chips to find an alternative for the Cheddar flavoring, the Emmental flavoring and Oaxaca flavoring.

Sample	Description	Result
Chips 2	As Chips 1 but with Cheddar-2 instead of the Cheddar flavoring (1:1)	Little more sour but close to the original
Chips 3	As Chips 1 but with Cheddar-1 instead of the Cheddar flavoring (1.4:1)	Sour, a little too low cheese taste but close to the original
Chips 4	As Chips 1 but with Cheddar-1 instead of the Cheddar flavoring (1.3:1)	Too low cheese taste
Chips 5	As Chips 1 but with Emmental-1 instead of the Emmental flavoring (1:1)	Nice chips, full-flavor taste.
Chips 6	As Chips 1 but with Oaxaca-1 and cream powder instead of Oaxaca flavoring (0.42:1 (oaxaca-1:oaxaca))	Coconut taste
Chips 7	As Chips 1 but with Oaxaca-1 instead of Oaxaca flavoring (0.5:1)	Feels like too much flavorings, and some off-taste
Chips 8	As Chips 1 but with Oaxaca-1 instead of Oaxaca flavoring (0.45:1)	Some off-taste
Chips 9	As Chips 1 but with Oaxaca-1 instead of Oaxaca flavoring (0.4:1)	Better than Chips 8 but still not as Chips 1
Chips 10	As Chips 1 but with Oaxaca-1 instead of Oaxaca flavoring (0.35:1)	More salty, high cheese, low sweet. No off-taste, but not as Chips 1
Chips 11	As Chips 1 but with Oaxaca-1 instead of Oaxaca flavoring (0.3:1)	Edgy, more sour, no deep in the taste, lack of body

When replacements had been developed for the cheese flavoring, they were tested together, table 8.

Table 8. Spice mixtures tested on chips after good alternatives for each flavoring was found.

Sample	Description	Result
Chips 12	As Chips 11 but also Cheddar-2 instead of the Cheddar flavoring (1:1) and Emmental-1 instead of the Emmental flavoring (1:1)	Low body
Chips 13	As Chips 1 but also Cheddar-2 instead of Cheddar flavoring (1:1) and Emmental-1 instead of Emmental flavoring (1:1) and Butter-2 instead of Oaxaca flavoring (0.08:1)	Pleasant taste, but a little low in body
Chips 14	As Chips 1 but also Cheddar-2 instead of Cheddar flavoring (1:1) and Emmental-1 instead of Emmental flavoring (1:1) and Butter-1 instead of Oaxaca flavoring (0.08:1)	Close to the original but less sour
Chips 15	As Chips 14 but Powder-1 instead of ordinary cheese powder (1:1)	High body, too much old cheese taste
Chips 16	As Chips 14 but Powder-2 instead of ordinary cheese powder (1:1)	Lower body than Chips 15
Chips 17	As Chips 14 but Powder-1 instead of ordinary cheese powder and flavoring Cheddar-2 (1:1)	Salty, not complete taste
Chips 18	As Chips 14 but Powder-2 instead of ordinary cheese powder and flavoring Cheddar-2 (1:1)	Too salty, not complete taste
Chips 19	As Chips 15 but 29 % lower amount of Cheddar-2	Top in the flavors, changed body
Chips 20	As Chips 14 but mixture of Powder-1 and Powder-2 instead of ordinary cheese powder, 80 % lower amount of Cheddar-2 (replaced with the two cheese powders) (1:1)	Low cheese, high chili/paprika
Chips 21	As Chips 14 but mixture of Powder-3 and ordinary cheese powder, 33 % lower amount of Emmental-1 (replaced with the two cheese powders) (1.5:1)	Too much butter taste
Chips 22	As Chips 14 but Powder-3 instead of ordinary cheese powder, 15 % lower amount of Cheddar-2 (replaced with Powder-3) (1:1)	Low flavor top
Chips 23	As Chips 14 but Powder-3 instead of ordinary cheese powder (1:1)	Close to the original
Chips 24	As Chips 23 but chili powder instead of Jalapeño-α flavoring (1:1)	Too much heat
Chips 25	As Chips 23 but chili powder and Jalapeño-1 instead of Jalapeño-α (1.5:1)	Tastes like jalapeño, not good
Chips 26	As Chips 25 but extra chili powder and lower Jalapeño-1 (1.5:1)	Tastes like jalapeño and is too hot
Chips 27	As Chips 24 but less chili powder (0.67:1)	Close to the original

When Chips 14 was developed a triangle sensory test was done on this chips together with the Original chips. The results are summarized in table 9. On a significance level of 5 % the test showed no difference between the two samples. For full results and calculations see Appendix II.

Table 9. Results from the triangle test of Chips 14 and Original chips.

Answer	Responses
Right	8
Wrong	9
Total	*17*

For the Chips 23 and Chips 27 a hedonic evaluation on the internal consumer panel was performed. The Chips 23 had been stored in room temperature for 3 days, and Chips 27 for 1 day prior analysis. The results were compared to Original chips stored in room temperature for 3 days prior analysis, analyzed at the same time as the two samples. The results are presented as box diagram with the minimum, first quartile, median, third quartile and the maximum values shown, figure 1-3. For the complete result see Appendix III. The mean values for each sample are shown in table 10. At the same occasion the panelists were asked to mark which tow samples that was the most similar, if not all three were very similar, figure 4.

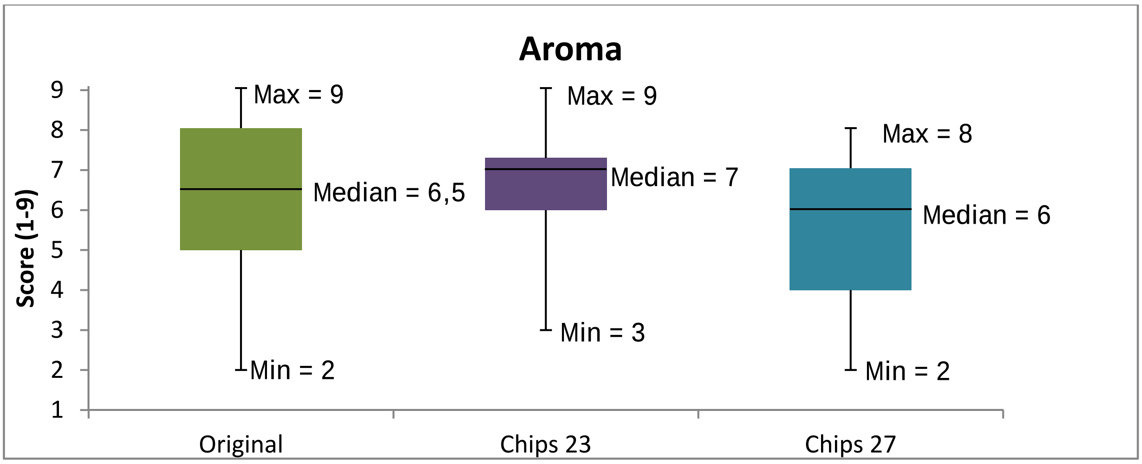

Figure 1. Results from the hedonic evaluation of the aroma in freshly opened bags of the Original chips, Chips 23 and Chips 27.

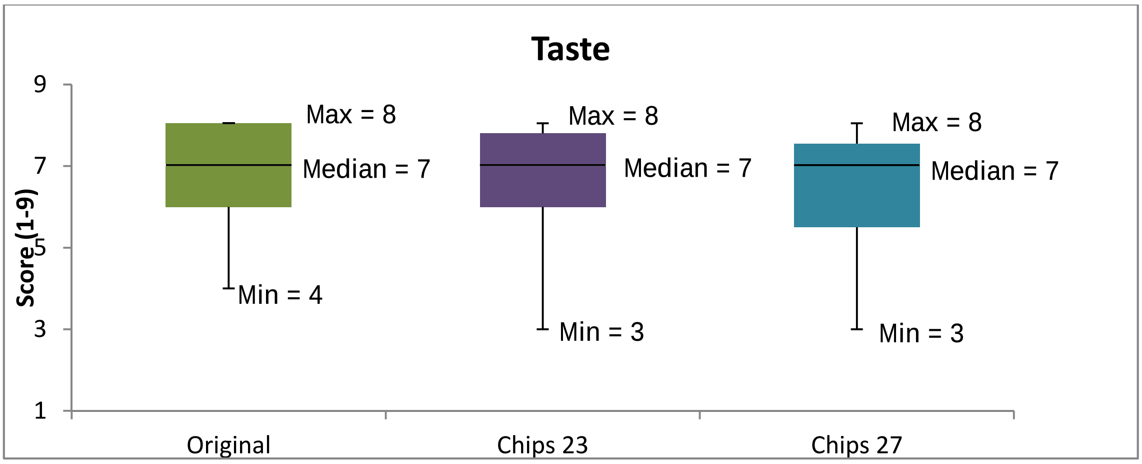

Figure 2. Results from the hedonic evaluation of the taste in freshly opened bags of the Original chips, Chips 23 and Chips 27.

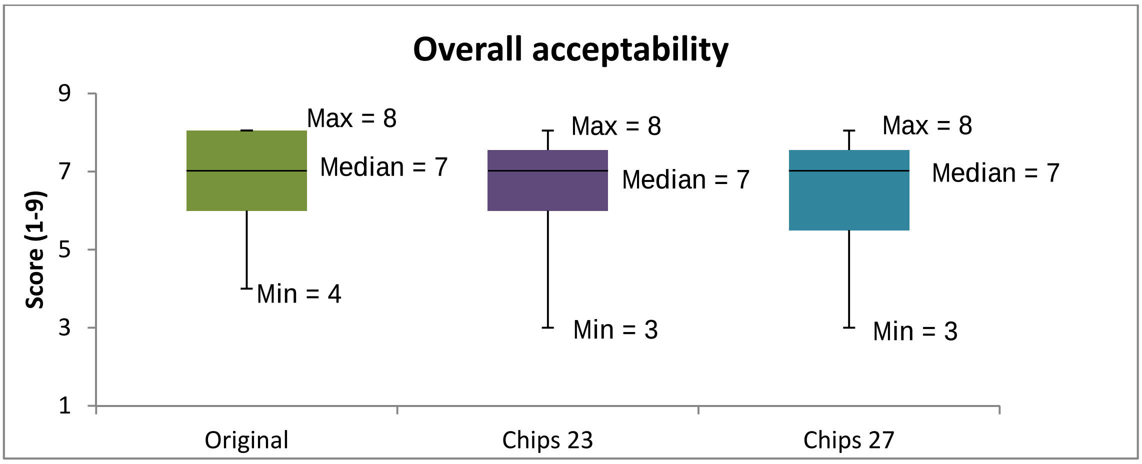

Figure 3. Results from the hedonic evaluation of the overall acceptability in freshly opened bags of the Original chips, Chips 23 and Chips 27.

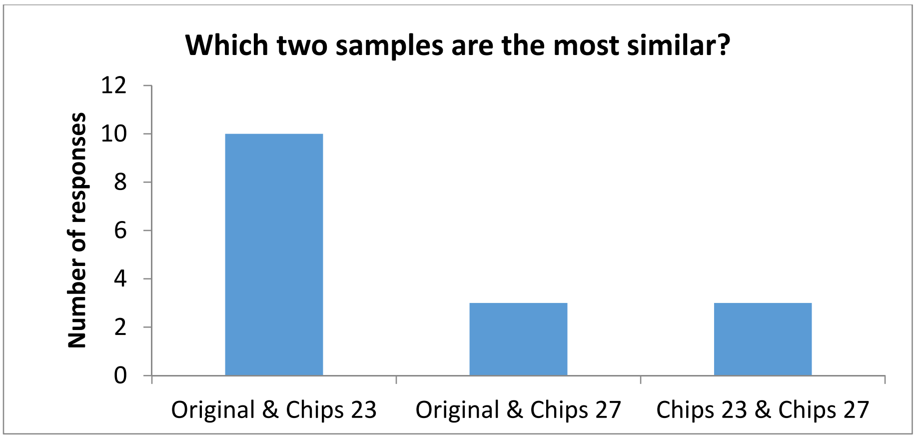

Figure 4. Results from the evaluation of which samples the panelists found most similar to each other.

Table 10. The mean values from the hedonic evaluation of Original chips, Chips 23 and Chips 27.

	Original	**Chips 23**	**Chips 27**
Aroma	6.25	6.70	5.65
Taste	6.84	6.56	6.56
Overall acceptability	6.79	6.63	6.42

The *t* test of the data showed no significant (α = 0.05) difference between Original chips and Chips 23 or between Original chips and Chips 27 for any of the tested attribute (Aroma, Taste and Overall acceptability). The result from the evaluation of the aroma of Chips 23 was compared to the same for Chips 27. The aroma of Chips 23 significantly (α = 0.05) differed from the aroma of Chips 27. See Appendix III for the calculations.

4.1.1 Product price Tortilla cheese chips

Due to secrecy the prices of the chips cannot be shown in fully, just as percentage of the Original chips. The production cost of the Chips 27 is approximately 0.53 % more expensive than the Original chips, calculated with buying the flavorings one by one, not a pre-prepared mixture of the Cheddar-2 and Emmental-1.

With a pre-prepared mixture of the Cheddar-2 and Emmental-1 (prepared from the distributer) the production cost of Chips 27 is approximately 0.16 % more expensive than the Original chips.

4.1.2 Declaration Tortilla cheese chips

Declaration of Original Tortilla cheese chips: Corn flour, sunflower/canola oil, salt, MILK powder, dextrose, yeast extract, sugar, flavorings (contains MILK), maltodextrin, onion, tomato, CHEESE powder (0.1%), acid (lactic acid), paprika extract, other spices.

Declaration of Chips 27: Corn flour, sunflower/canola oil, salt, MILK powder, dextrose, maltodextrin, sugar, yeast extract, natural flavorings (contains MILK), onion, tomato, CHEESE powder (0.1%), acid (lactic acid), paprika extract, other spices.

4.2 Product development – Cheese dip mix

For the Cheese dip mix, several different mixtures were tested by two to five R&D specialists at Santa Maria AB. A description of respectively dip are presented in table 11 together with the results from the tasting.

Table 11. The different dips tested. Each dip is compared to the original dip mix.

Sample	Description	Results
Dip 1	Cheddar, Emmental and Cheese booster flavorings replaced as in the cheese chips	Less cheese taste, more sour.
Dip 2	As Dip 1 but without Jalapeño-β	Straggling taste. Feels incomplete
Dip 3	Same as Dip 2 but without lactic acid	Feels the yeast extract taste
Dip 4	As Dip 1 but with additional cheese powder, type Powder-3	Full-flavored cheese taste with some sharp cheese taste
Dip 5	A Dip 1 but only with Powder-3	Full-flavored cheese taste
Dip 6	As Dip 5 but Jalapeño-β replaced with jalapeño-1 and lower amount Yeast flavoring	Mild, nice taste
Dip 7	As Dip 6, addition of chili powder	Interesting with the extra hotness
Dip 8	As Dip 5 but lower amount of Yeast flavoring	The lower amount of Yeast flavoring is better than the higher used in Dip 6
Dip 9	As Dip 6 but extra Jalapeño-1 and lower amount Yeast flavoring	Too much jalapeño taste
Dip 10	As Dip 9 but less sugar	Balanced, and pleasant taste
Dip 11	As Dip 10 but addition of Jalapeño-α	Pleasant with the hotness
Dip 12	As Dip 10 but less Emmental-1 and more Powder-3	Milder, less interesting than Dip 11
Dip 13	As Dip 10 but without lactic acid	No difference without the lactic acid.
Dip 14	As Dip 10 but without Yeast flavoring	Missed the umami taste
Dip 15	As Dip 11 but without lactic acid	No difference compared to Dip 12
Dip 16	As Dip 15 but less Emmental-1	Pleasant, full-flavored cheese taste
Dip 17	As Dip 16 but Jalapeño-α replaced with chili powder.	Hot, pleasant cheese taste, good!
Dip 18	As Dip 17 but with the same proportion of Cheddar-1/Emmental-1 as in the Chips 27	A bit weak in cheese taste, but still a pleasant Cheese dip

Dip 13 and Dip 17 was evaluated as good alternatives to the original dip. Both these two were evaluated in a hedonic evaluation together with the original dip. The results are presented as box diagram with the minimum, first quartile, median, third quartile and the maximum values shown, figure 5-7. For the complete result and calculations see Appendix IV. The mean values for each sample are shown in table 12.

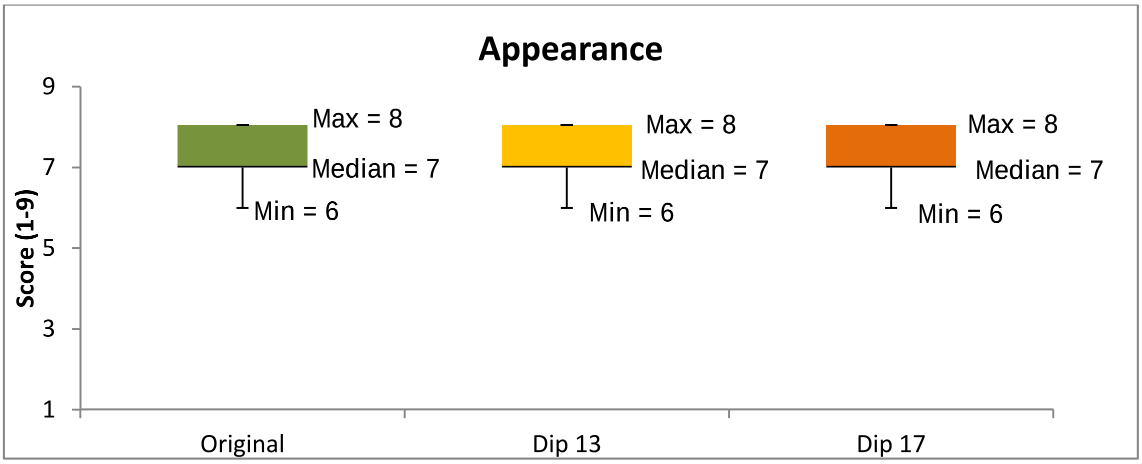

Figure 5. Results from the hedonic evaluation of the appearance of the Original dip, Dip 13 and Dip 17.

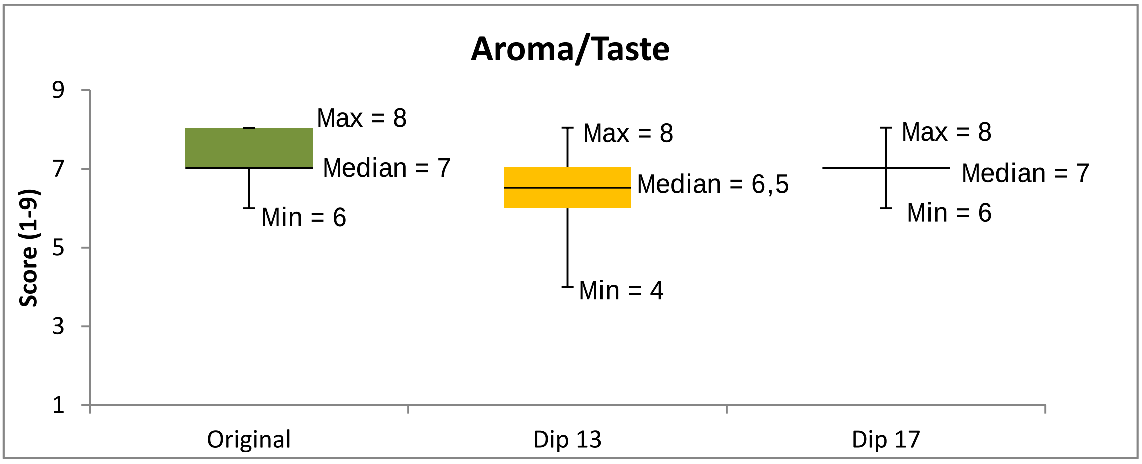

Figure 6. Results from the hedonic evaluation of the aroma/taste of the Original dip, Dip 13 and Dip 17.

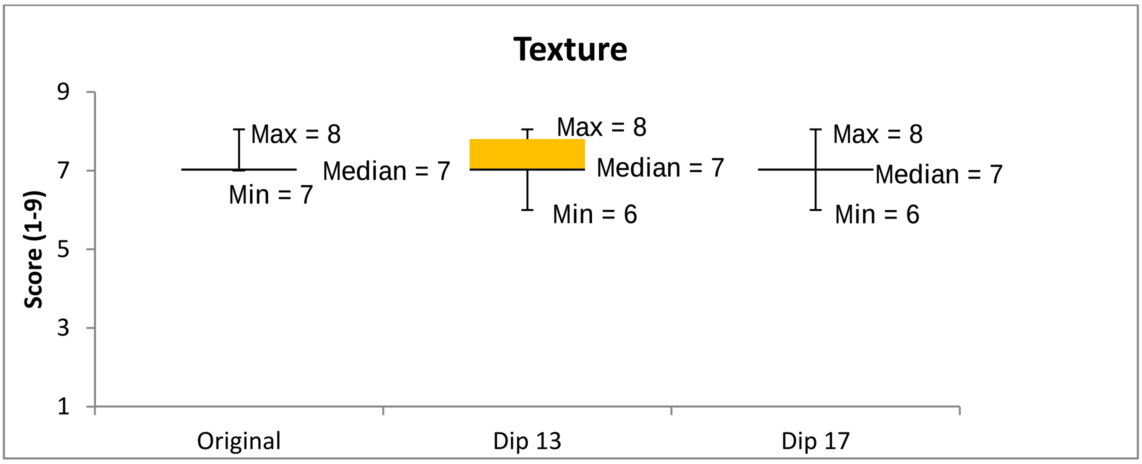

Figure 7. Results from the hedonic evaluation of the texture of the Original dip, Dip 13 and Dip 17.

Table 12. The mean values from the hedonic evaluation of Original dip, Dip 13 and Dip 17.

	Original	Dip 13	Dip 17
Appearance	7.3	7.3	7.3
Aroma/Taste	7.2	6.4	7.0
Texture	7.2	7.1	7.0

The *t* test of the data showed no significant (α = 0.05) difference between Original dip and Dip 14 or between Original dip and Dip 17 for any of the tested attribute (Appearance, Aroma/Taste and Texture). See Appendix VI for the calculations.

The Dip 17 showed highest result of the two dips with NA flavorings tested. To ensure this was a good alternative one more hedonic evaluation of this dip was performed, this time with more panelists. At the same occasion Dip 18 was tested, as well as the Original dip. For complete results see Appendix V. Below, in figure 8-10, are the results presented as box diagram with the minimum, first quartile, median, third quartile and the maximum values shown. The mean values for each sample are shown in table 13.

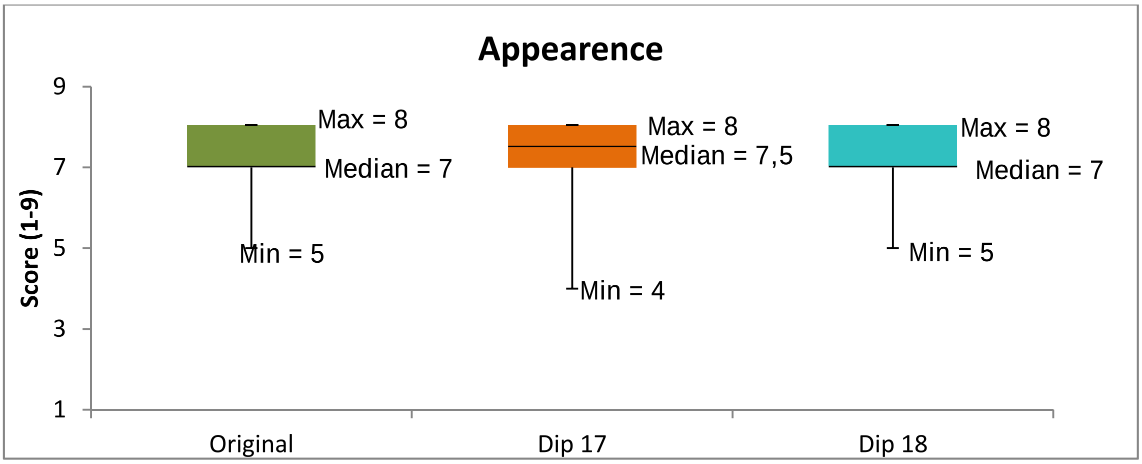

Figure 8. Results from the hedonic evaluation of the appearence re of the Original dip, Dip 17 and Dip 18.

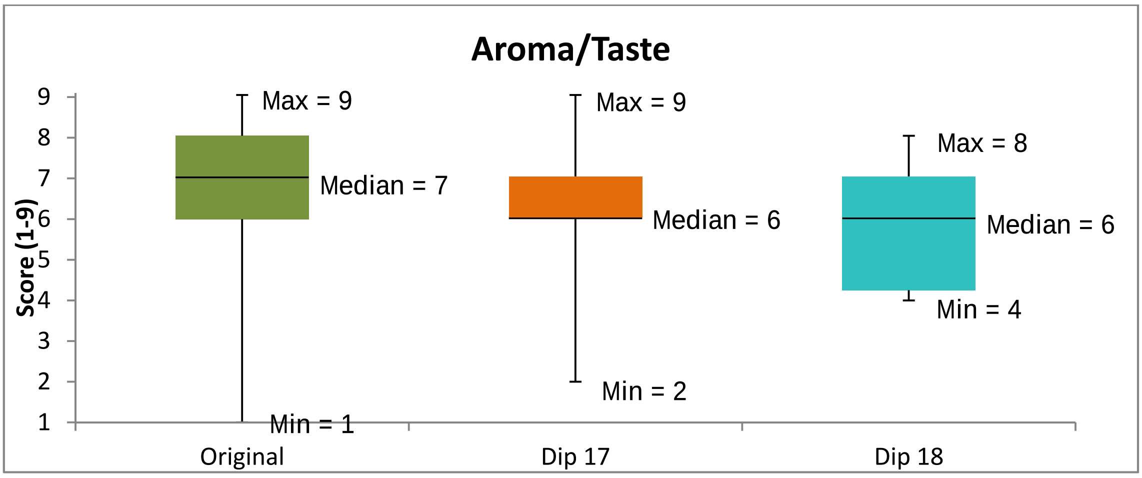

Figure 9. Results from the hedonic evaluation of the aroma/taste re of the Original dip, Dip 17 and Dip 18.

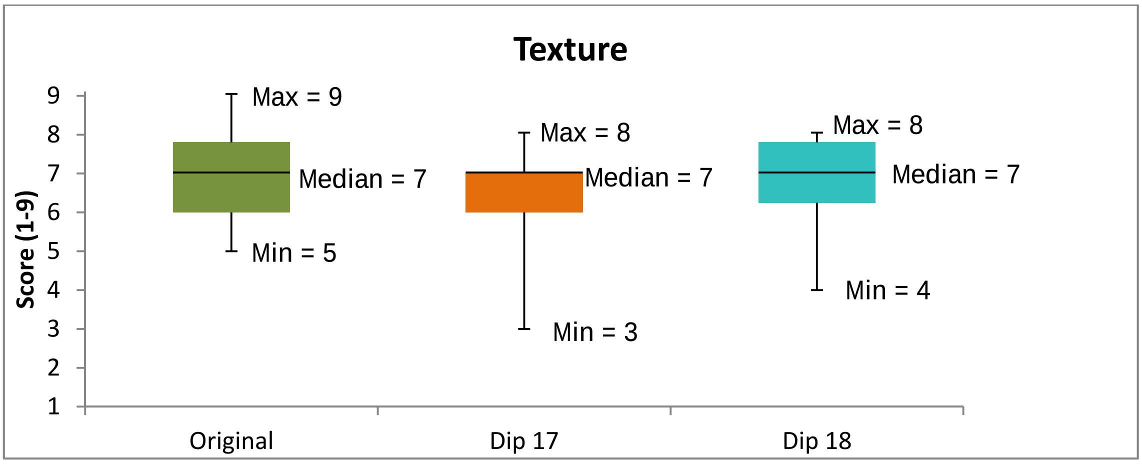

Figure 10. Results from the hedonic evaluation of the texture of the Original dip, Dip 17 and Dip 18.

Table 13. The mean values from the hedonic evaluation of Original dip, Dip 17 and Dip 18.

	Original	Dip 17	Dip 18
Appearance	7.2	7.1	7,2
Aroma/Taste	6.6	6.4	5,8
Texture	6.8	6.6	6,8

The *t* test of the data showed no significant (α = 0.05) difference between Original dip and Dip 17 or between Original dip and Dip 18 for any of the tested attribute (Appearance, Aroma/Taste and Texture). See Appendix V for the calculations.

4.2.1 Product price cheese dip mix

Due to secrecy the prices of the dips cannot be shown in fully, just as percentage of the Original dip. The production cost of Dip 17 is approximately 0.45 % higher than the Original dip calculated with buying the flavorings one by one, not a pre-prepared mixture of the Cheddar-2 and Emmental-1.

If using the same proportion between the two NA cheese flavorings as in the Tortilla cheese chips, it would be possible to buy a pre-prepared mixture of Cheddar-2 and Emmental-1 from the distributer, (prepared from the distributer). This was done in Dip 18. The production cost of Dip 18 was approximately 0.39 % higher than the Original dip.

4.2.2 Declaration Cheese dip mix

Declaration Original Cheese dip mix: Maltodextrin, MILK powder, salt, CHEESE powder (11%), sugar, flavorings (contains MILK), onion, crushed chili, color (paprika extract), turmeric, thickener (E412), jalapeño pepper, anti-caking agent (E551)

Declaration Dip 17 (1): Maltodextrin, MILK powder, CHEESE powder (13%), salt, sugar, natural flavorings (contains MILK), onion, crushed chili, color (paprika extract), turmeric, thickener (E412), jalapeño pepper, anti-caking agent (E551)

Declaration Dip 17 (2): Maltodextrin, MILK powder, CHEESE powder (13%), salt, sugar, natural jalapeño flavoring with other natural flavorings (contains MILK), onion, crushed chili, color (paprika extract), turmeric, thickener (E412), jalapeño pepper, anti-caking agent (E551)

The declaration for Dip 18 would be the same as for Dip 17.

4.3 Accelerated shelf life test

The results from both Method 1 and Method 2 are presented in this part.

4.3.1 Method 1

For Method 1 the chips were analyzed after two and a half weeks. At that point one sample had been in 42°C for one week and then this sample had been in the freezer (-20°C). The other samples were in the climate cabinet until a half week before analysis and at that point they were put into the freezer(-20°C). The results are presented in figure 11. During the test the temperature in the climate cabinets and the room was measured with a separate thermometer, the results from this are shown in Appendix VIII.

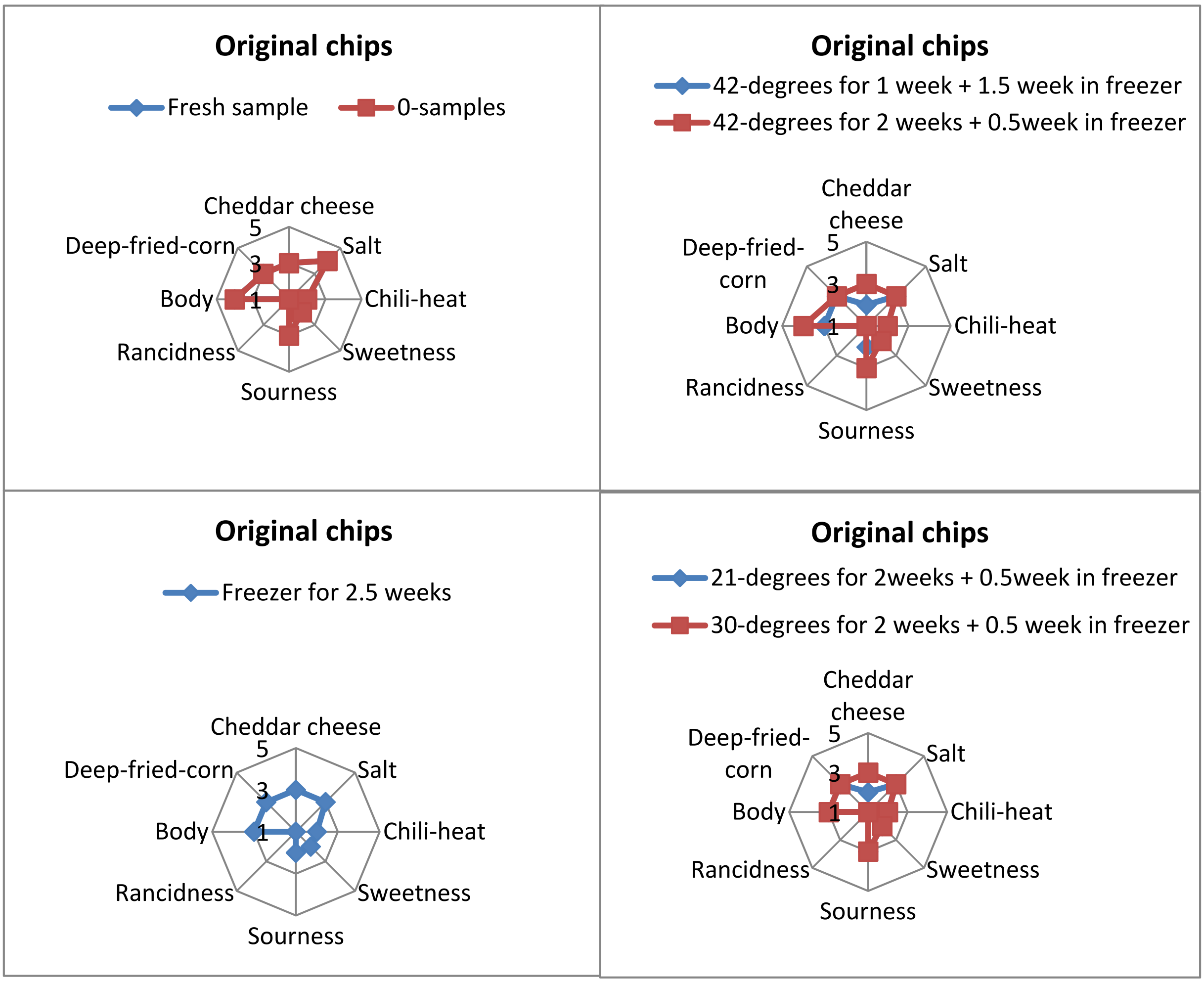

Figure 11. The screening profile for; Upper left: Fresh sample and 0-sample. Upper right: Sample stored in 42°C for one respectively two weeks, and in freezer (-20°C) for remaining time until total two and a half week. Lower left: sample stored in freezer (-20°C) for two and a half weeks. Lower right: Samples stored in climate cabinet (21°C and 30°C respectively) for two weeks and then in freezer (-20°C) for a half week.

4.3.2 Method 2

For Method 2 spice mixture and chips were analyzed for both Original and Chips 14. The results are presented in this part separately for spice mixture and chips. During the test the temperature in the climate cabinets was measured with a separate thermometer, the results from this are shown in Appendix VIII. For the parameters chosen for the evaluation, the results were tested if they followed zero or first order reaction. Since almost all seemed to follow zero order this was chosen for all, see Appendix IX.

4.3.2.1 Spice mixture

The spice mixtures were stored in three different temperatures, 30°C, 42°C and 50°C. During the storage the color and texture of the spice mixtures were highly affected by the temperature and storage time. In figure 12 are freshly made, 20 days in storage and 50 days in storage Original spice mixture shown for all three storage temperatures. The samples stored in 50°C had a burnt color after 50 days and was clumpy/hard/dry in texture. In 42°C the samples became darker and darker as the storage time increased, but not as burnt color as the 50°C samples. Also the texture became more and more clumpy/hard/dry but not in the same extends as the 50°C samples. The 30°C samples were similar in both color and texture to the freshly made spice mixture.

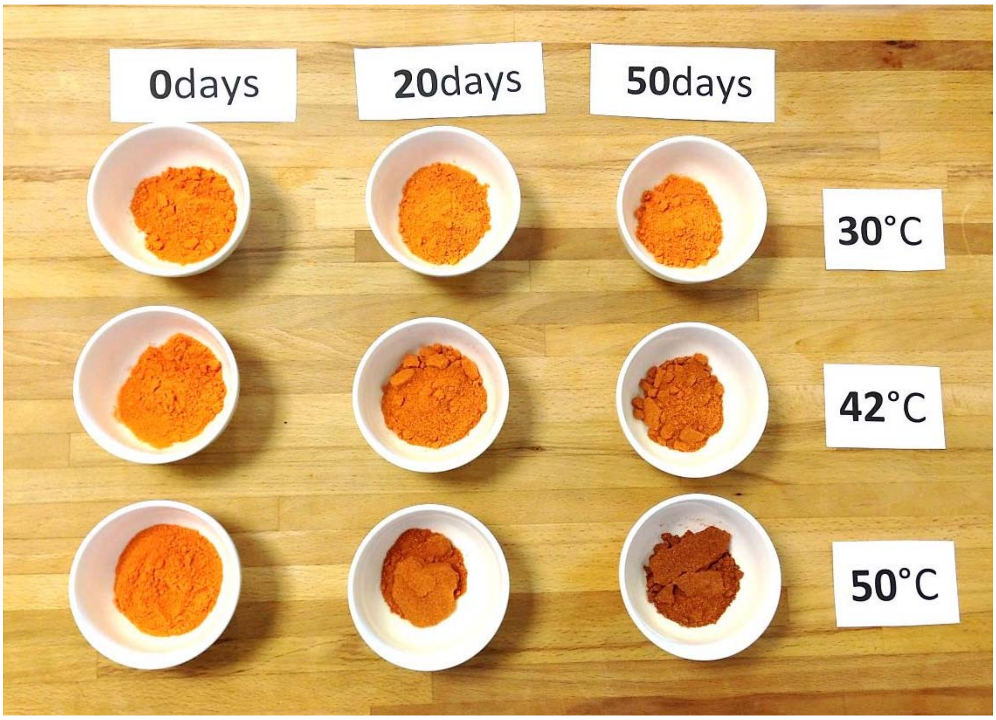

Figure 12. Storage samples of Original spice mixtures. Two samples for each temperature, after 20 respectively 50 days of storage, as well as freshly made spice mixture.

To evaluate if the amount of air the spice mixture was exposed to affected the results, a four times as big bag, compared to the other samples, was filled with the same amount of spice mixture. The sample was stored in 42°C and was evaluated after 50 days. The same was done for both spice mixture of Original and Chips 14. Both "Big bag" samples were similar in appearance. The color was lighter than the sample stored in the same temperature for the same time period but in a smaller bag. The color was more close to the freshly made spice mixture. Also the texture was more close to the freshly made spice mixture, it was not clumpy/hard/dry. The two samples stored in 42°C for 50 days, one in big bag and one in a small bag, are shown in figure 13 together with fresh spice mixture.

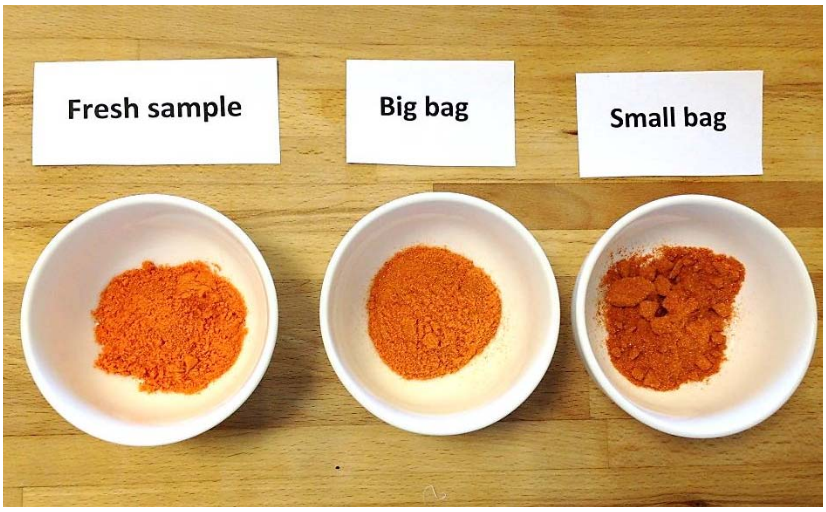

Figure 13. Fresh spice mixture together with the two samples stored in 42°C for 50 days, one in big bag and one in a small bag.

The screening profile of the big bag sample was slightly different compared to the small bag sample, stored at the same temperature the same time. The biggest difference was that the small bag had considerably higher ‘Burnt cauliflower smell’. The small bag had a five (the highest) for both the Original and the Chips 14, whilst the big bag had a two for the Original spice mixture and a three for the Chips 14 spice mixture, figure 14 shows the screening profiles of the big bags and small bags for both the Original spice mixture and the Chips 14 spice mixture.

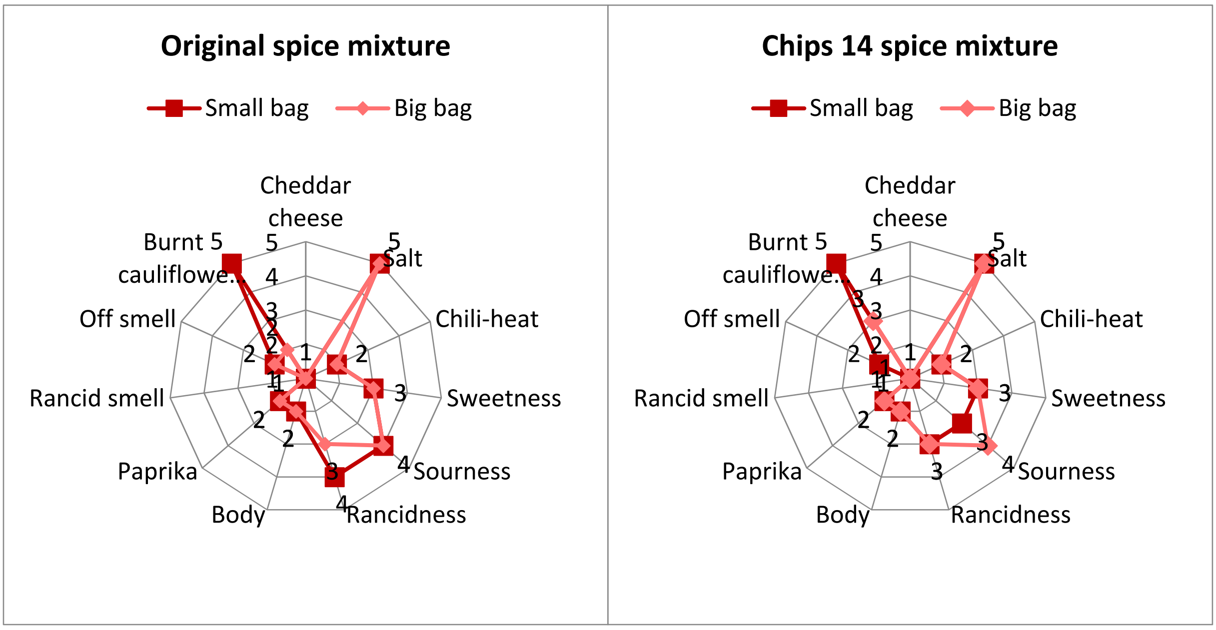

Figure 14. Screening profiles for samples stored in a small bag (same as all other storage samples) and a big bag (4 times as big) in 42°C for 50 days (same amount of spice mixture in both samples) for both the Original spice mixture and the Chips 14 spice mixture.

For the screening of the spice mixtures, it was four of the parameters that was changed the most during the storage; ‘Cheddar-cheese’, ‘Rancidness’, ‘Off smell’ and ‘Burnt cauliflower smell’. Of these four the two taste parameters (‘Cheddar-cheese’ and ‘Rancidness’) were chosen for calculations of the activation energy and reaction constants since they showed more stable results than the aroma parameters. For results of all measured parameters see Appendix X. The results from the two aroma parameters are shown in figure 15-16.

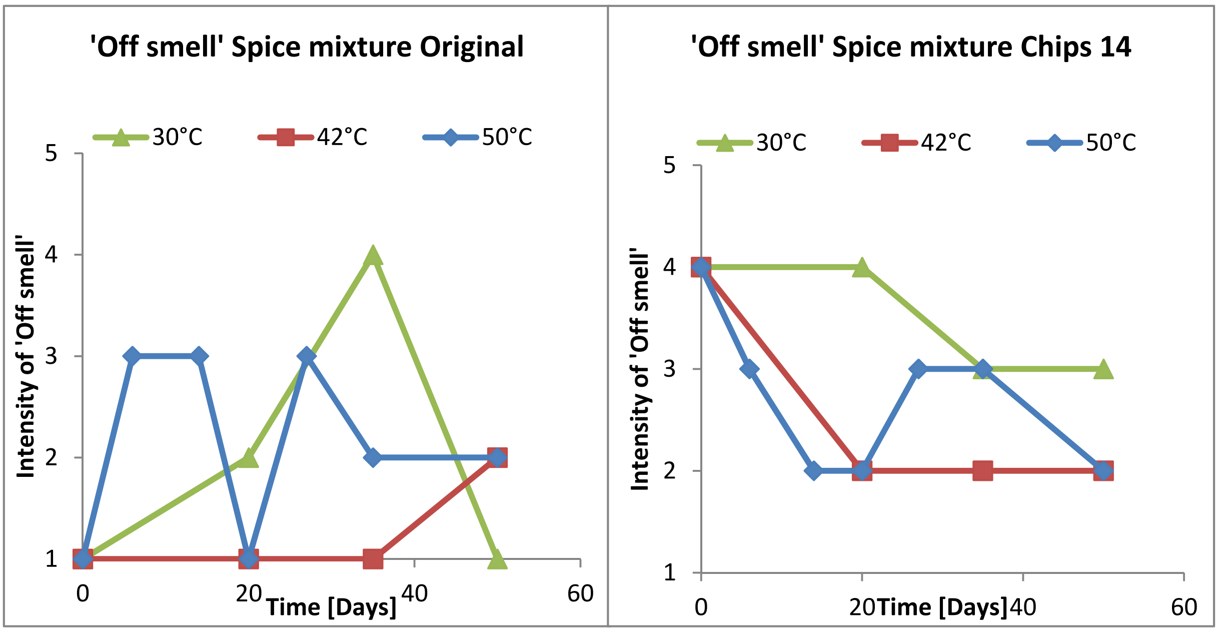

Figure 15. Intensity of 'Off smell' in the tested samples for both Original spice mixture and Chips 14 spice mixture.

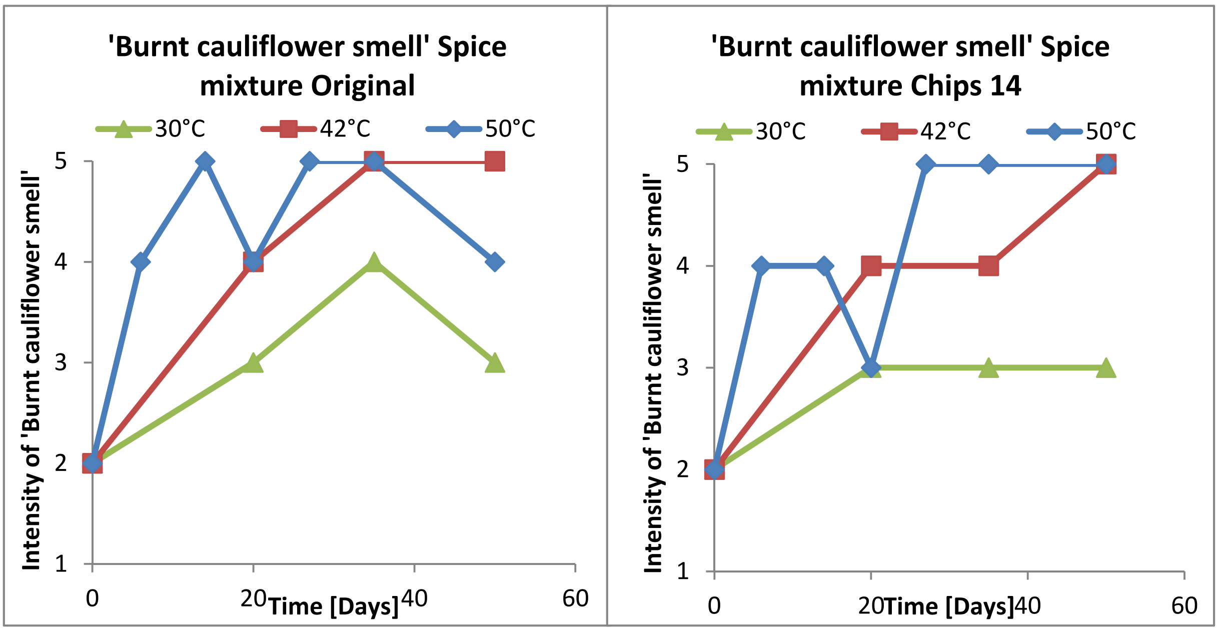

Figure 16. Intensity of 'Burnt cauliflower smell' in the tested samples from both Original spice mixture and Chips 14 spice mixture.

To evaluate the method, and calculate a corresponding time in room temperature of the Chips 14, the reaction constants for the two parameters ‘Cheddar-cheese’ and ‘Rancidness’ was calculated for each test temperature. The natural logarithm of these constants were plotted against the time in a so called Arrhenius plot to estimate the reaction constant in room temperature for each of the two parameters.

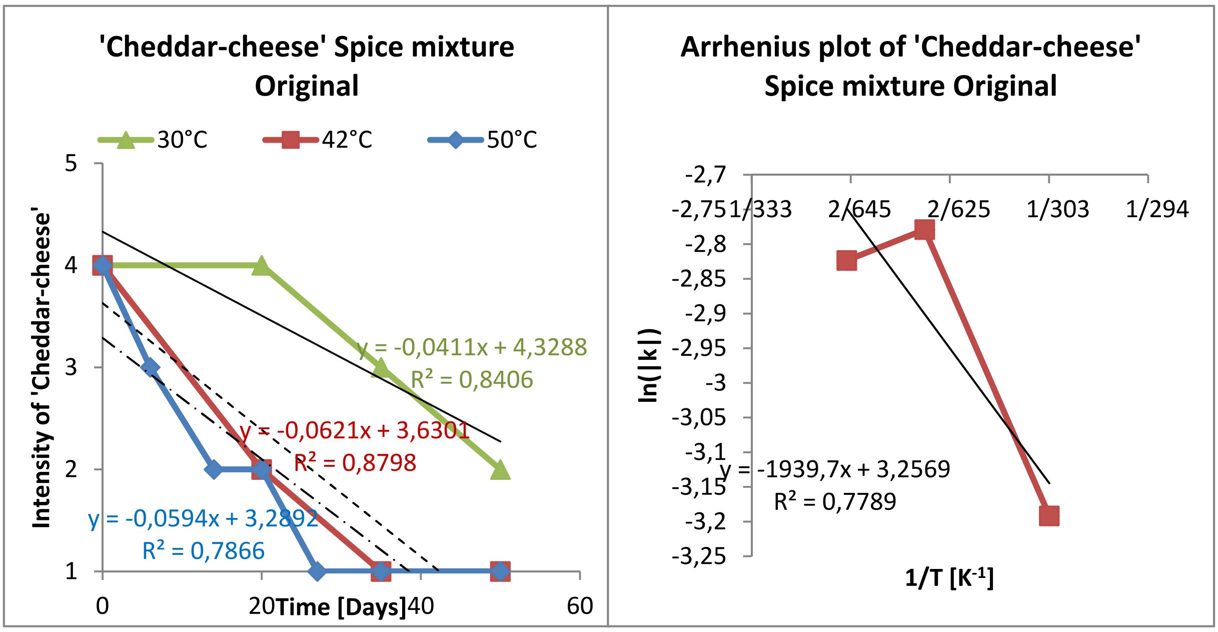

Figure 17. To the left: The intensity of 'Cheddar-cheese' for Original spice mixture is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

For the Original spice mixture the parameter 'Cheddar-cheese' seems to decrease rapider with increasing temperature and time, this can be seen in figure 17 in the increasing slope of the trend lines. The natural logarithm of the obtained k-values (negative the slope of the trend line in left figure 17) was plotted against the inverse temperature in a so called Arrhenius plot. From this the activation energy was calculated, see table 14. By extrapolating the trend line in the Arrhenius plot to room temperature (295 K) the reaction constant for room temperature (295 K) was obtained, table 14, and this value was compared to the reaction rates at the different test temperatures to see how much the temperature raised the reaction time, table 14.

The same as above was made for the parameter 'Rancidness' for the Original spice mixture, and both the parameters 'Cheddar-cheese' and 'Rancidness' for Chips 14 spice mixture. The activation energy, k-value at room temperature and reaction rate comparison for each temperature was also obtained as explained above and can be found in table 14. The intensity graphs and corresponding Arrhenius plots are shown in figure 18-20.

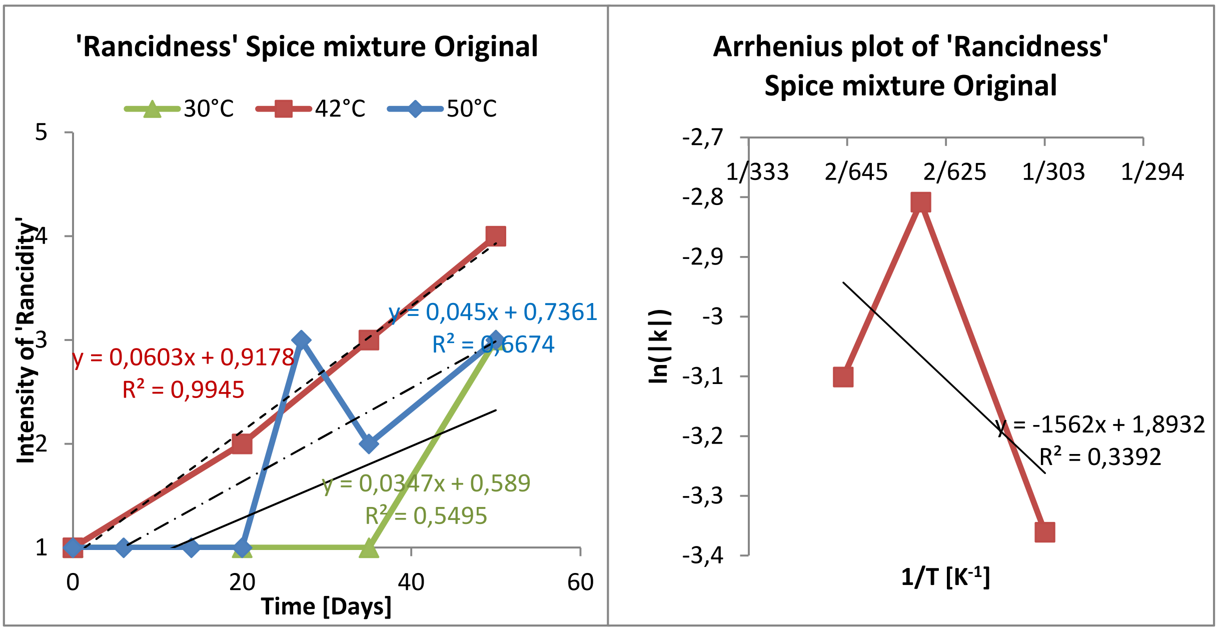

Figure 18. To the left: The intensity of 'Rancidness' for Original spice mixture is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

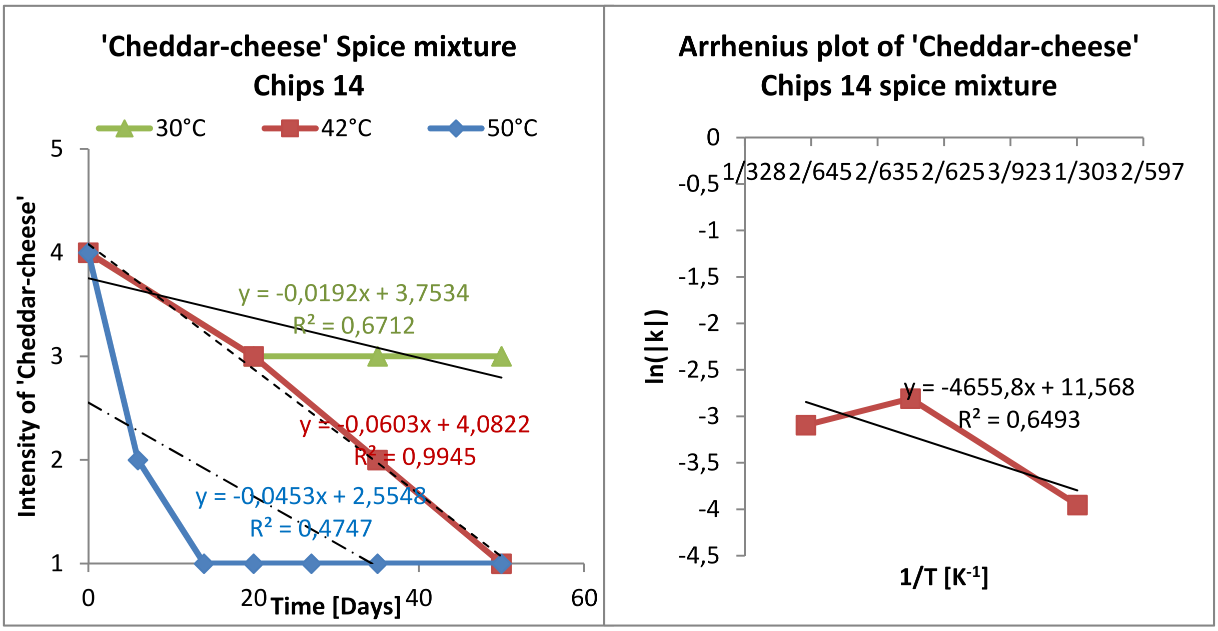

Figure 19. To the left: The intensity of 'Cheddar-cheese' for Chips 14 spice mixture is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

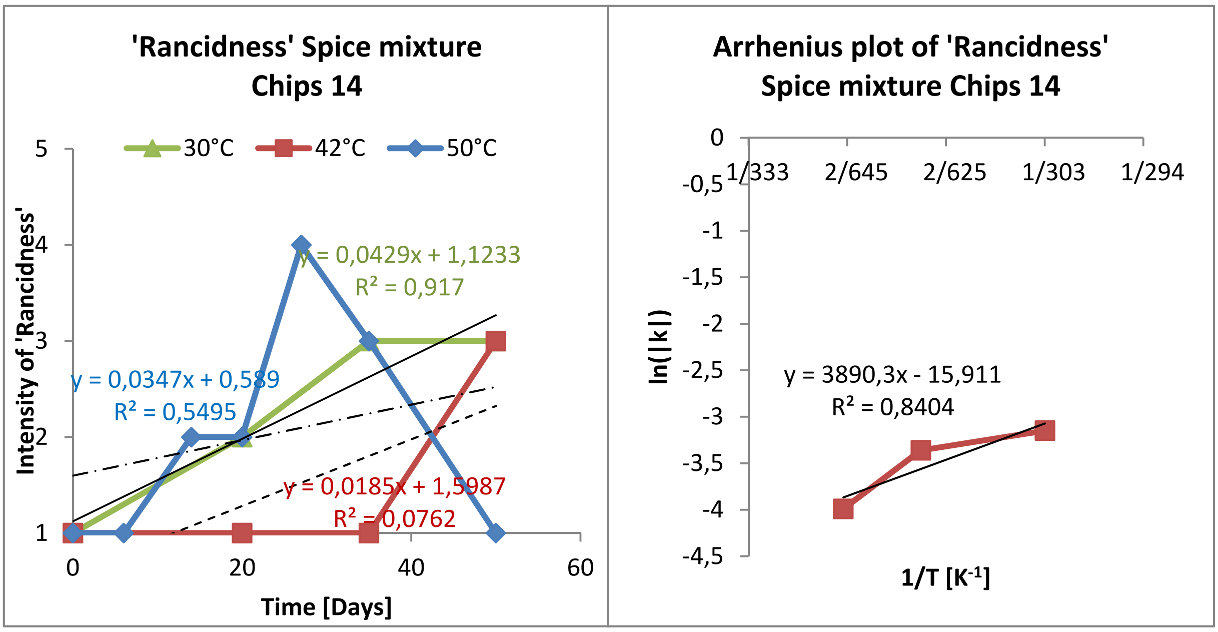

Figure 20. To the left: The intensity of 'Rancidness' for Chips 14 spice mixture is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

Table 14. Activation energy, reaction constant (k) at room temperature and reaction rate comparison, all for the parameters 'Cheddar-cheese' and 'Rancidness' for both the Original spice mixture and Chips 14 spice mixture.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature
'Cheddar-cheese' Spice mixture Original	3.85	0.036	1.13 time as high at 30°C
			1.71 times as high at 42°C
			1.64 times as high at 50°C
'Rancidness' Spice mixture Original	3.10	0.033	1.04 time as high at 30°C
			1.81 times as high at 42°C
			1.35 times as high at 50°C
'Cheddar-cheese' Spice mixture Chips 14	9.25	0.015	1.30 time as high at 30°C
			4.08 times as high at 42°C
			3.06 times as high at 50°C
'Rancidness' Spice mixture Chips 14	-7.73	0.066	0.65 time as high at 30°C
			0.53 times as high at 42°C
			0.28 times as high at 50°C

It is possible to do the calculations in different ways, by not using all points when calculating the reactions constants. One way is to start the calculation when the reaction starts and end when the reaction seems to end. When using this method it is important to choose the points to use with great care since it is possible to show misleading results if starting to late and ending to early. One example is if the score only goes from a one and to a two and not higher. Then it is important to take enough one-points to get valid results, and not only the last one point and the first two point, then the result is only dependent on the sampling time. For the spice mixtures the results for the parameter 'Rancidness' was calculated by using this method, figure 21-22 and table 15.

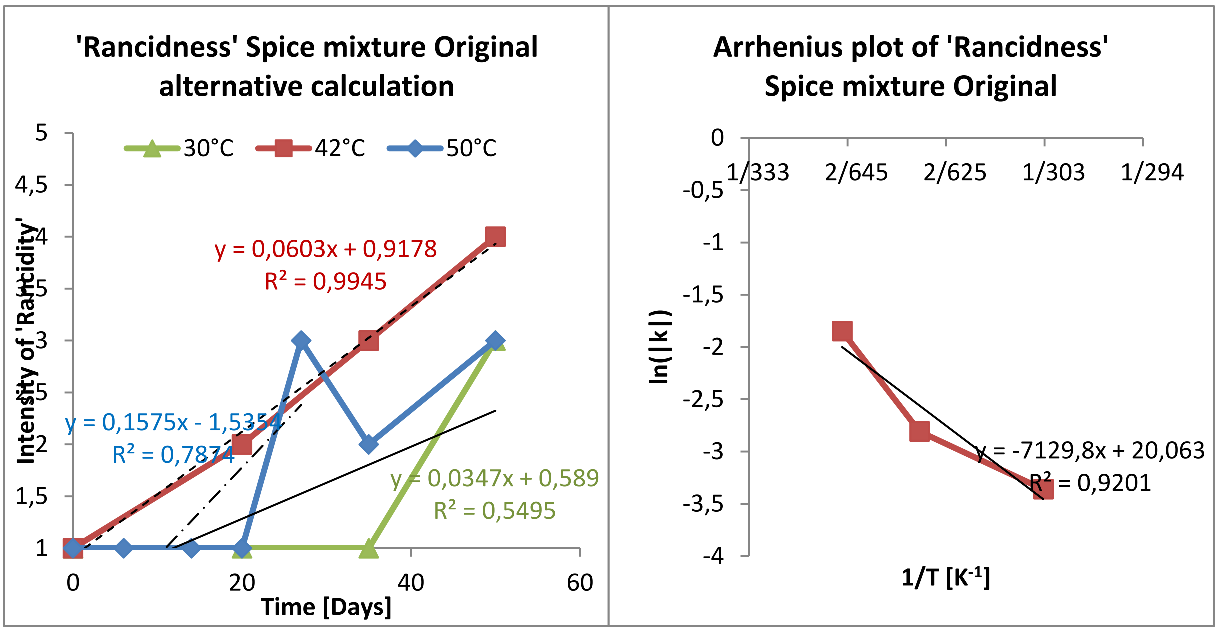

Figure 21. To the left: The intensity of ’Rancidness’ for Original spice mixture is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature, calculated by using; for 30°C: all points, for 42°C: all points and for 50°C: the second to fifth points. To the right: The corresponding Arrhenius plot.

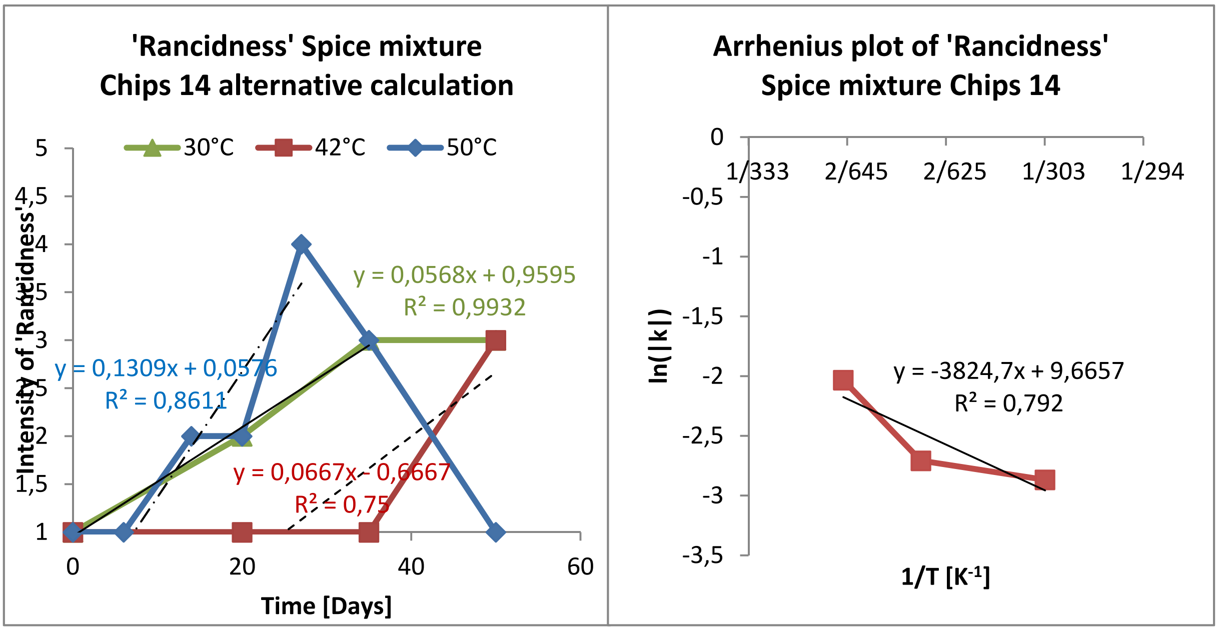

Figure 22. To the left: The intensity of ’Rancidness’ for Chips 14 spice mixture is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature, calculated by using; for 30°C: first three points, for 42°C: last three points and for 50°C: the second to fifth points. To the right: The corresponding Arrhenius plot.

Table 15. Activation energy, reaction constant (k) at room temperature and reaction rate comparison, all for the parameters 'Rancidness' when doing the alternative calculation for both the Original spice mixture and Chips 14 spice mixture.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature
'Rancidness' Spice mixture Original alternative calculation	14.2	0.016	2.11 time as high at 30°C
			3.66 times as high at 42°C
			9.56 times as high at 50°C
'Rancidness' Spice mixture Chips 14 alternative calculation	7,6	0.037	1.54 time as high at 30°C
			1.81 times as high at 42°C
			3.55 times as high at 50°C

The weight difference, after storage compared to before, of each sample was measured. The results are presented in figure 23 for both the Original spice mixture and the Chips 14 spice mixture.

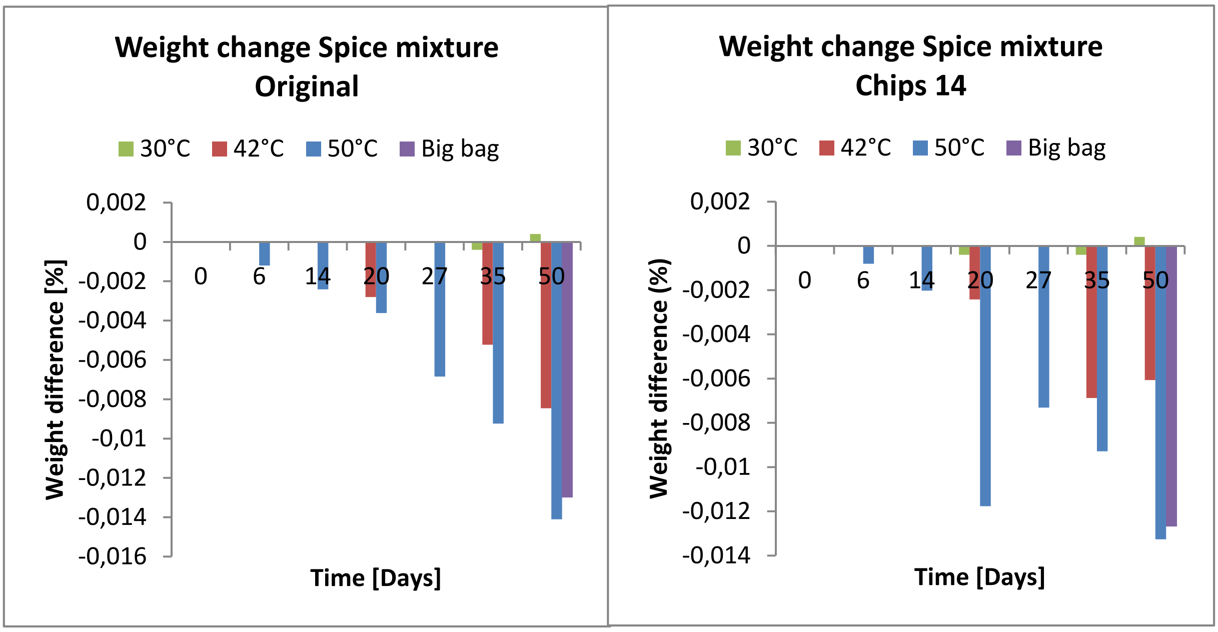

Figure 23. Sample weight difference, after storage compared to before, for the three different test temperatures. To the left: the Original spice mixture. To the right: the Chips 14 spice mixture. In purple is the "Big bag" samples shown. They were stored in 42°C

4.3.2.2 Chips

For the screening of the chips, it was four of the parameters that changed the most during the storage; 'Cheddar-cheese', 'Rancidness', 'Off smell' and 'Rancid smell'. Of these four the two taste parameters ('Cheddar-cheese' and 'Rancidness') were chosen for calculations of the activation energy, reaction constant and shelf life since they showed more stable results than the aroma parameters. For results of all measured parameters see Appendix X. The results from the two aroma parameters are shown in figure 24-25.

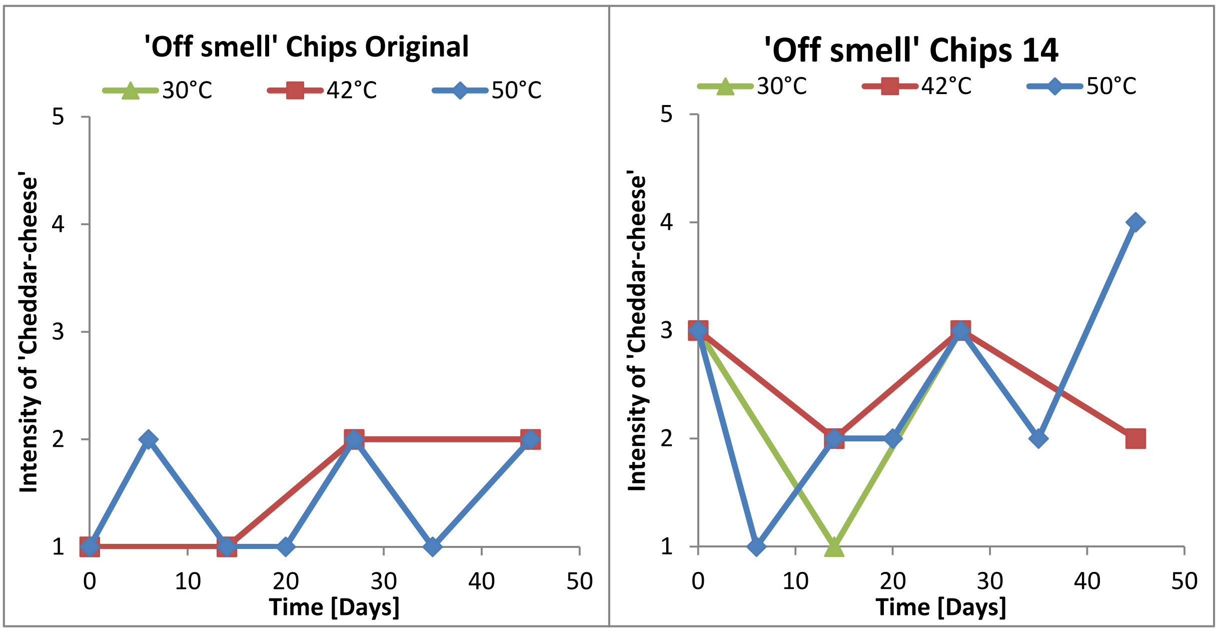

Figure 24. Intensity of 'Off smell' in the tested samples for both Original chips and Chips 14.

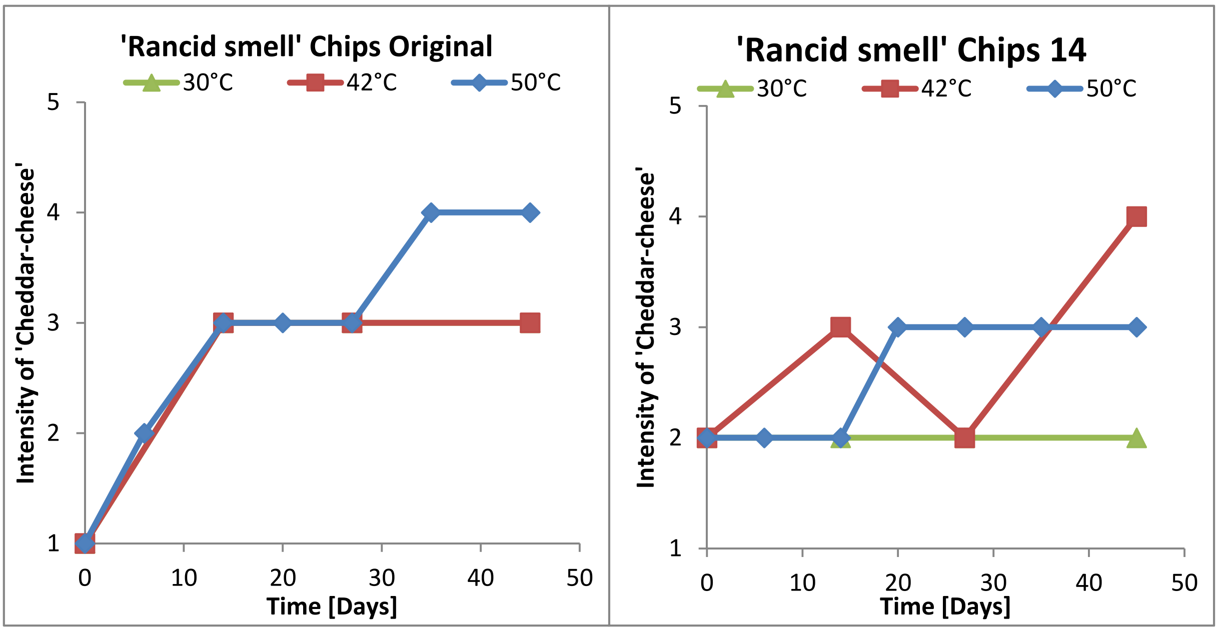

Figure 25. Intensity of 'Rancid smell' in the tested samples for both Original chips and Chips 14.

To evaluate the shelf life, and evaluate the method, expired chips were analyzed. The screening profile of these chips can be seen in figure 26. The parameters changed the most from fresh chips are the 'Rancid smell', 'Cheddar-cheese' and 'Rancidness'. To calculate the shelf life of the product the parameter 'Rancidness' was chosen since of those three parameters that is the one consumers are most unwilling to eat. Hence, the chips have expired when the rancidness is evaluated as a four on the used five grade scale.

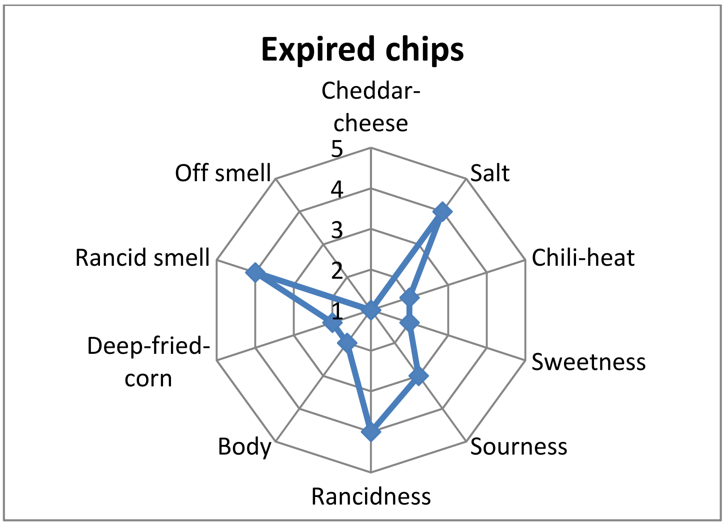

Figure 26. Screening profile of expired chips (26 days over expire date).

For the parameter 'Cheddar-cheese' the activation energy, k-value at room temperature and reaction rate comparison for each temperature were calculated as for the spice mixture. The intensity of the parameter and the corresponding Arrhenius plots can be found in figure 27-28. The obtained activation energy, k-value at room temperature and reaction rate comparison for each temperature can be found in table 16 for both Original chips and Chips 14.

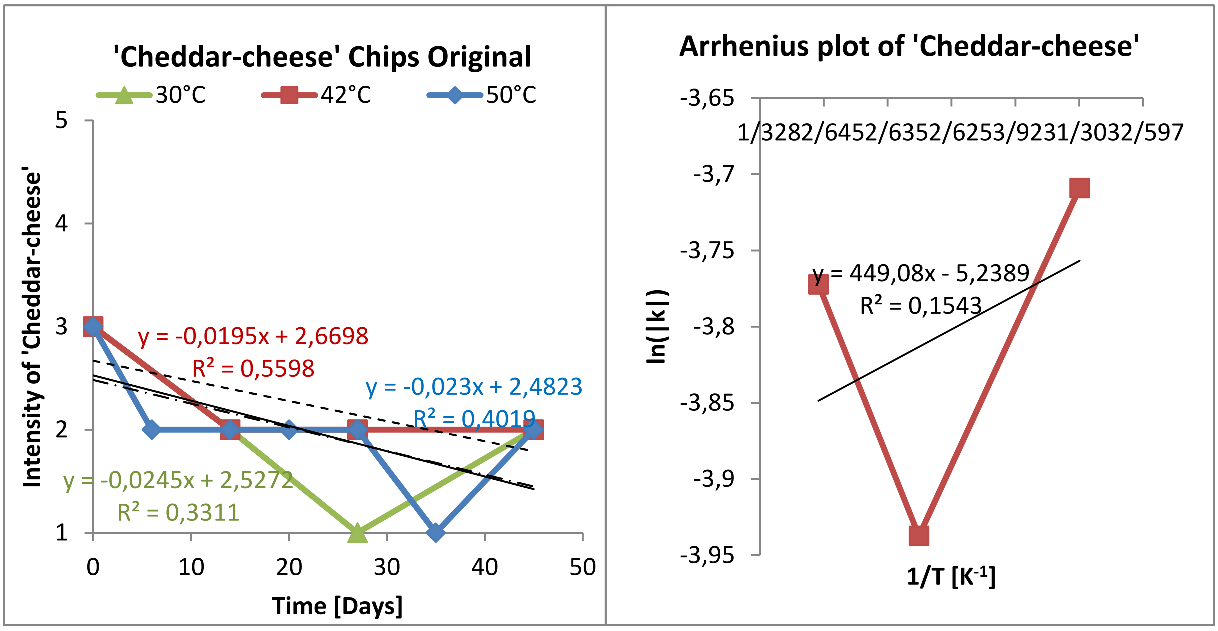

Figure 27. To the left: The intensity of 'Cheddar-cheese' for Original chips is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

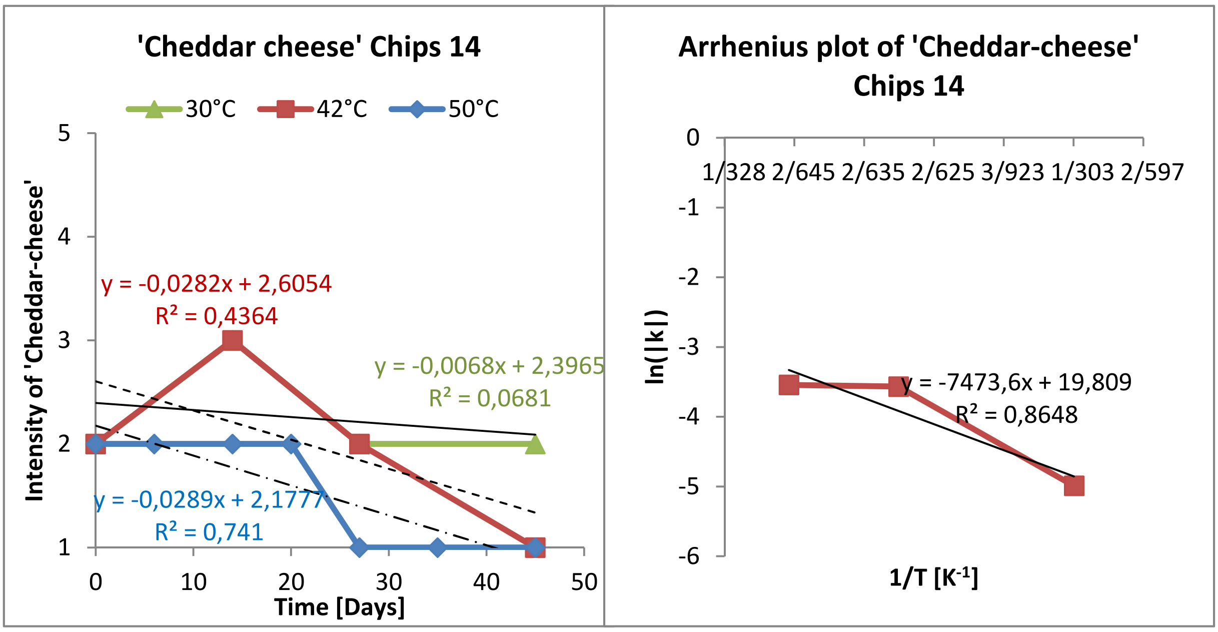

Figure 28. To the left: The intensity of 'Cheddar-cheese' for Chips 14 is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

Table 16. Activation energy, reaction constant (k) at room temperature and reaction rate comparison, all for the parameter 'Cheddar-cheese' for both the Original chips and Chips 14.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature
'Cheddar-cheese' Original chips	-0.89	0.024	1.01 time as high at 30°C
			0.80 times as high at 42°C
			0.95 times as high at 50°C
'Cheddar-cheese' Chips 14	14.85	0.004	1.71 time as high at 30°C
			7.08 times as high at 42°C
			7.25 times as high at 50°C

For the parameter 'Rancidness' the intensity graph and corresponding Arrhenius plot can be found in figure 29 for the original chips, and in figure 30 for the Chips 14. For each of the two, the Original chips and the Chips 14, the shelf life was calculated by calculation of how long time that was necessary in each temperature to reach an intensity of 'Rancidness' of four and how long time this corresponds to in room temperature (295 K). The activation energy, k-value at room temperature, reaction rate comparison for each temperature and the shelf life are presented in table 17 for both the Original chips and Chips 14.

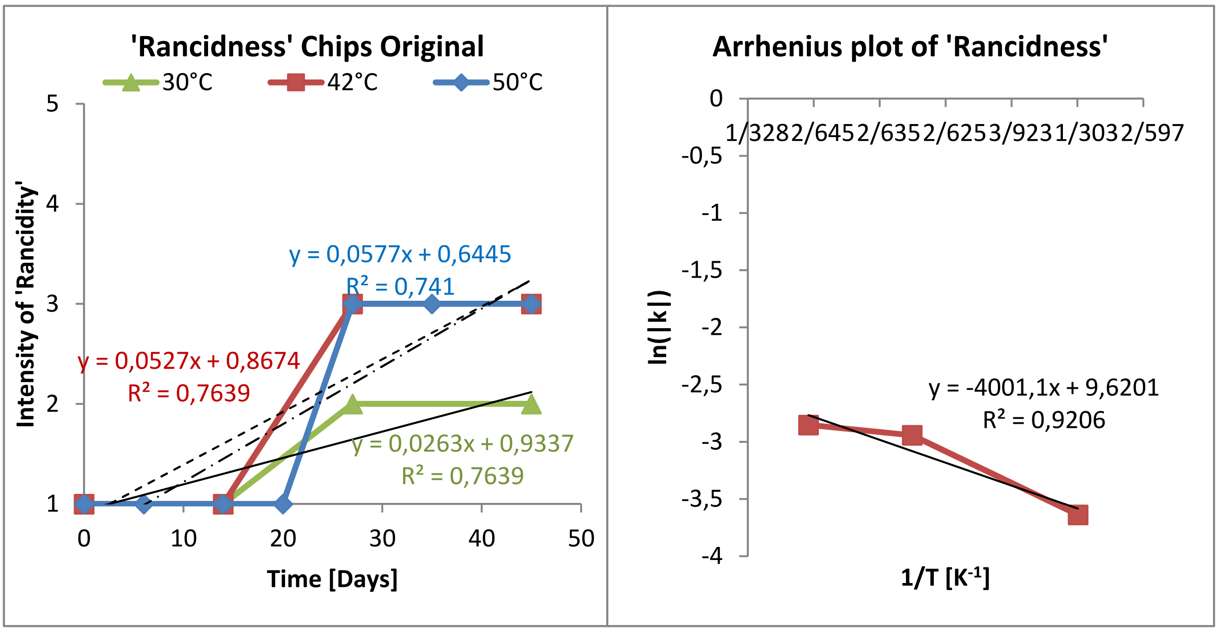

Figure 29. To the left: The intensity of 'Rancidness' for the Original chips is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

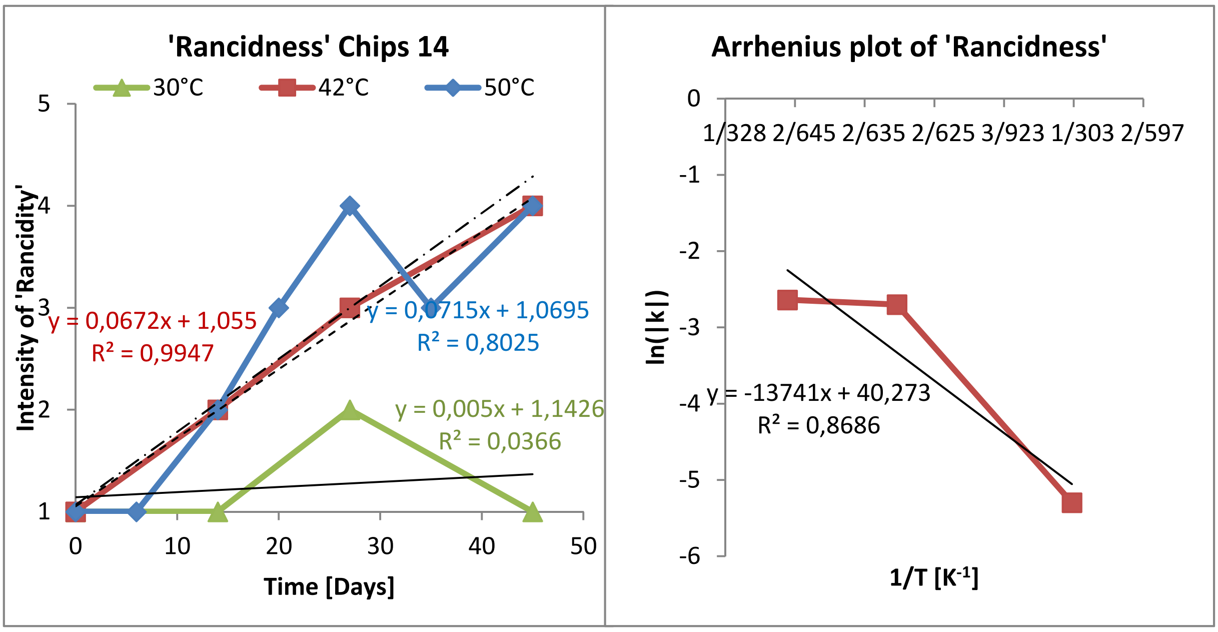

Figure 30. To the left: The intensity of 'Rancidness' for the Chips 14 is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

Table 17. Activation energy, reaction constant (k) at room temperature, reaction rate comparison and calculated shelf life at room temperature, all for the parameter 'Rancidness' for both the Original chips and Chips 14.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature	Shelf life in room temperature [months]	Mean shelf life [months]
'Rancidness' Original chips	7.95	0.019	1.35 time as high at 30°C	5.2	5.4
			2.72 times as high at 42°C	5.3	
			2.98 times as high at 50°C	5.7	
'Rancidness' Chips 14	27.3	0.0018	2.74 time as high at 30°C	51.5	52.5
			36.84 times as high at 42°C	53.1	
			39.20 times as high at 50°C	52.8	

For the Chips 14 a shelf life of above 50 months was obtained. This is an unrealistic value since the shelf life would then be almost six times as high as for the shelf life for Original chips, which is nine months in real time. This may be due to a miss evaluation of the 'Rancidness' parameter for the last sample of the 30°Csamples. An additional calculation of the shelf life of Chips 14 was made with the assumption that the last point for Chips 14 at 30°Cwould be two, see results, together with the activation energy, the k-value at room temperature and the reaction rate comparison for each temperature, in table 18.

Table 18. Activation energy, reaction constant (k) at room temperature, reaction rate comparison and calculated shelf life at room temperature, all for the parameter 'Rancidness' for Chips 14 with corrected value of 'Rancidness' for the last 30°C sample.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature	Shelf life in room temperature [months]	Mean shelf life [months]
'Rancidness' Chips 14	14.6	0.013	1.95 time as high at 30°C	7.5 (30°C)	7.3
			4.98 times as high at 42°C	7.2 (42°C)	
			5.30 times as high at 50°C	7.1 (50°C)	

It is possible to calculate the shelf life in different ways from the data. For example, one can only look on the data until the samples in one of the temperatures show consistent results. This was done for both the Original and Chips 14, parameter 'Rancidness', figure 31-32 and table 19 Another way to do the calculation is to exclude the time until the reaction starts to happen and also when the reaction ends, as was done for the spice mixtures. This was done also done for the chips, parameter 'Rancidness', figure 33-34 and table 20.

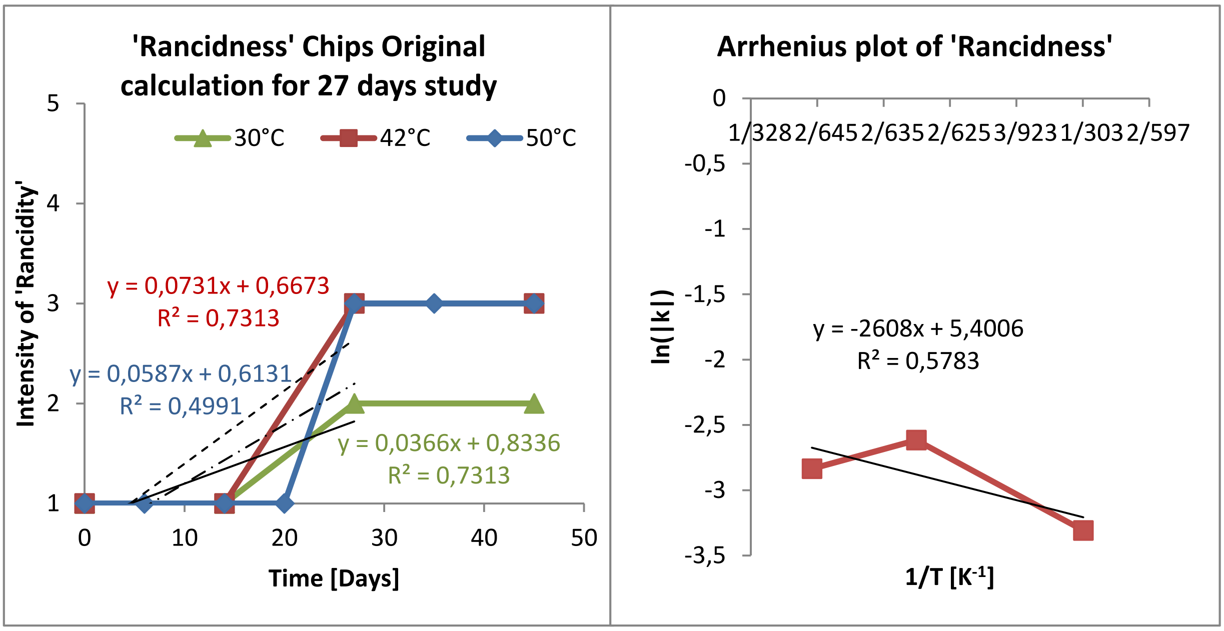

Figure 31. To the left: The intensity of 'Rancidness' for the Original chips is plotted against the time in days. The linear trend line for the first samples, up to day 27, as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

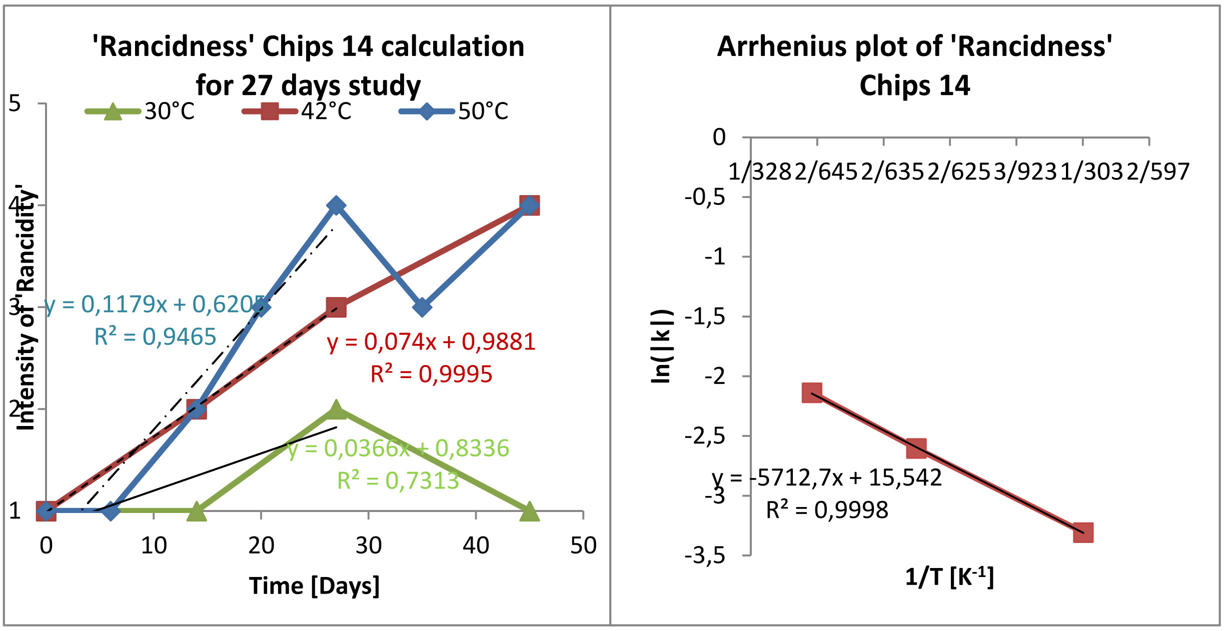

Figure 32. To the left: The intensity of 'Rancidness' for the Chips 14 is plotted against the time in days. The linear trend line for the first samples, up to day 27, as well as the correlation factor R^2 can be found in the graph for each temperature. To the right: The corresponding Arrhenius plot.

Table 19. Activation energy, reaction constant (k) at room temperature, reaction rate comparison and calculated shelf life at room temperature, all for the parameter 'Rancidness' for the Original chips and Chips 14. Only the samples up to 27 days from start are used for the calculations.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature	Shelf life in room temperature [months]	Mean shelf life [months]
'Rancidness' Original chips – samples first 27days	5.2	0.032	1.14 time as high at 30°C	3.25	3.4
			2.28 times as high at 42°C	3.42	
			1.83 times as high at 50°C	3.47	
'Rancidness' Chips 14 – samples first 27days	11.4	0.022	1.67 time as high at 30°C	4.76	4.8
			3.39 times as high at 42°C	4.53	
			5.39 times as high at 50°C	5.09	

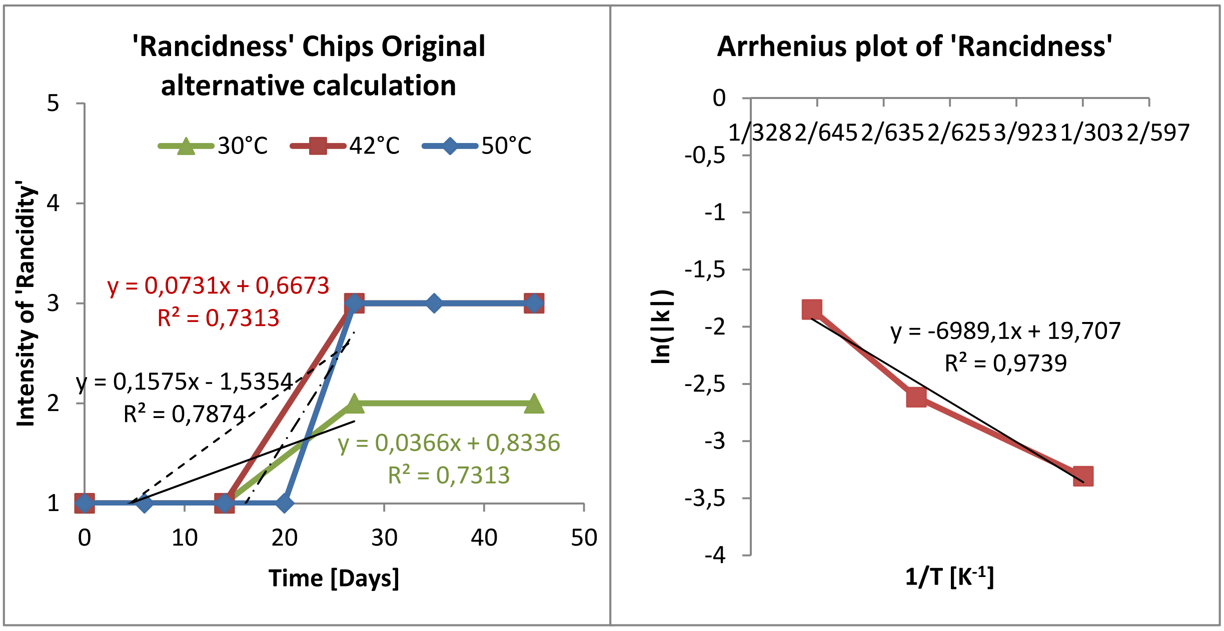

Figure 33. To the left: The intensity of 'Rancidness' for Original chips is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature, calculated by using; for 30°C: first three points, for 42°C: first three points and for 50°C: the third to fifth points. To the right: The corresponding Arrhenius plot.

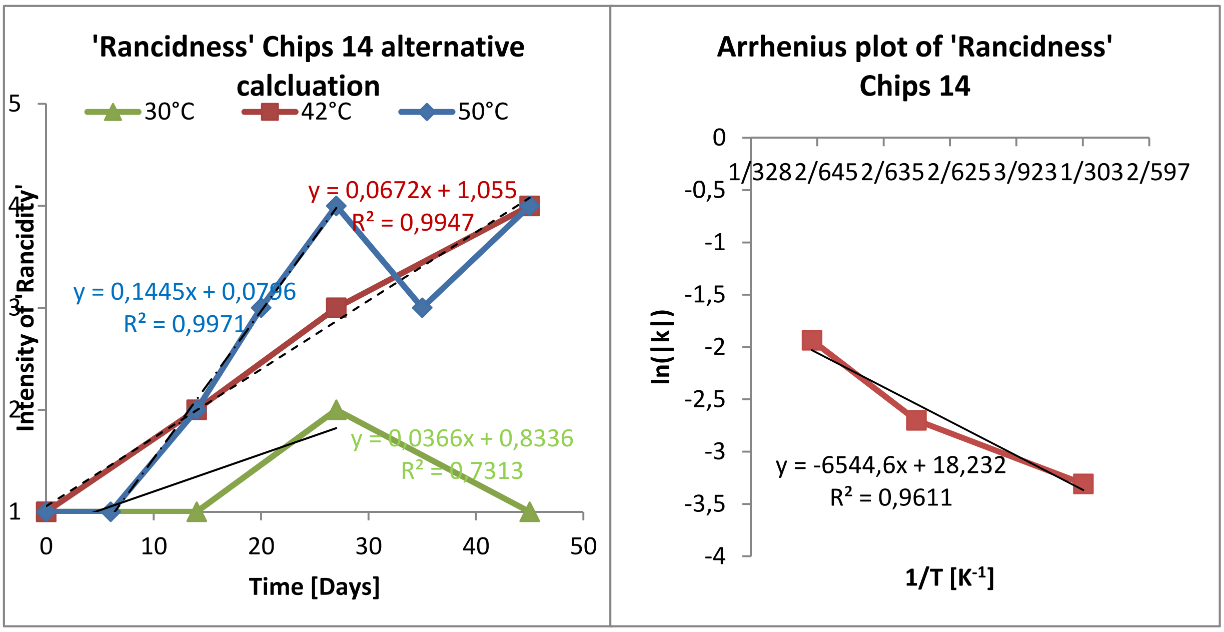

Figure 34. To the left: The intensity of 'Rancidness' for Original chips is plotted against the time in days. The linear trend line as well as the correlation factor R^2 can be found in the graph for each temperature, calculated by using; for 30°C: first three points, for 42°C: all points and for 50°C: the second to fifth points. To the right: The corresponding Arrhenius plot.

Table 20. Activation energy, reaction constant (k) at room temperature and reaction rate comparison, all for the parameters 'Rancidness' when doing the alternative calculation for both the Original chips and Chips 14.

Samples	Activation energy [kcal/(mole)]	k-value at room temperature [295 K]	Reaction rate compared to at room temperature	Shelf life in room temperature [months]	Mean shelf life [months]
'Rancidness' Original chips – alternative calculation 2	13.9	0.019	1.97 time as high at 30°C	5.60	7.1
			3.93 times as high at 42°C	5.90	
			8.47 times as high at 50°C	9.79	
'Rancidness' Chips 14 – alternative calculation 2	13.0	0.019	1.91 time as high at 30°C	5.43	5.7
			3.50 times as high at 42°C	5.05	
			7.53 times as high at 50°C	6.72	

The weight difference, after storage compared to before, of each sample was measured. The results are presented in figure 35 for both the Original chips and the Chips 14.

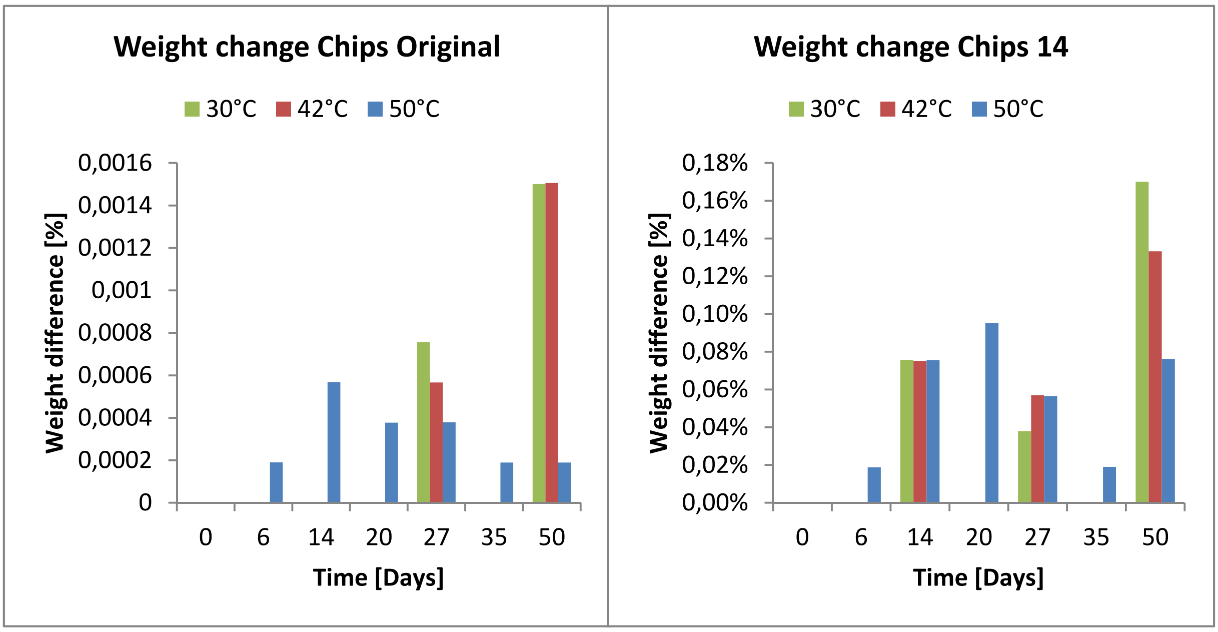

Figure 35. Sample weight difference, after storage compared to before, for the three different test temperatures. To the left: the Original chips. To the right: the Chips 14.

5 Discussion

The food industry strives to replace synthetic flavorings with natural (NA) ones since this is an increasing demand from the consumers. This projects focus was to do the replacement in two of Santa Maria's products, Tortilla cheese chips and Cheese dip mix. Alongside with the replacement process a method for accelerated shelf life study of seasoned tortilla chips was developed.

5.1 Product development

In this project, only two of the six synthetic flavorings were found to have a match in the NA flavorings available from the distributer. All could not be found since all synthetic flavorings cannot be produced as NA flavorings with the same properties due to several different reasons. The reasons can be that the flavoring molecules cannot be found in nature, the NA flavoring is not clean enough from toxins or the process required for the production is not approved for producing NA flavorings. The last one is a main problem according to one of the distributers of flavorings for this project, which wishes to stay anonymous, since several popular flavorings are produced during cooking of food, and one of the not approved processes is high heat treatment (over 120°C). Another problem is the price of the flavoring. According to the same distributer, it is likely that most flavorings can be produced as NA ones but for several of them it would be too expensive to develop and produce them.

As mentioned above, two of the synthetic flavorings, used in both the Tortilla cheese chips and the Cheese dip mix, could be replaced with NA flavorings without using anything else than one NA flavoring for each of them. Two other synthetic flavorings, one in the Tortilla cheese chips and one in the Cheese dip mix, could be replaced with NA flavoring combined with other raw materials, and the last flavorings was replaced with only other raw materials (not flavorings). So to replace synthetic flavorings a lot of work is needed to both find NA flavorings with a pleasant taste and smell, and if this cannot be obtained, find other alternatives for the replacement. If the price of the product cannot be raised it is also necessary to complement the NA flavorings with other, cheaper, raw materials.

During this project, it was discovered that one of the NA flavorings (Emmental-1) made the Tortilla cheese chips smell in a way that some consumers found unpleasant. The Cheese dip mix did not have the same problem, probably since the product is consumed in sour milk and the smell is capsulated. The smell when opening a bag of Tortilla cheese chips was examined by the internal consumer panel. The test was done for the Original chips, stored in room temperature for three days (the minimum time it takes from production to a consumer in reality), Chips 23 (containing the Emmental-1 flavoring) also stored in room temperature for three days and Chips 27 (containing the Emmetnal-1 flavoring) stored one day in room temperature. For further description of Chips 23 and Chips 27, see table 8. For the results, see figure 1-3. For neither the Chips 23 nor the Chips 27, the aroma did not significantly ($\alpha = 0.05$) differ from the Original chips, but the aroma of Chips 23 significantly ($\alpha = 0.05$) differed from the one of Chips 27. In the comments from the test, not presented in this report, it appeared that the Chips 27 smelled more artificial than the Chips 23. Earlier the smell of Emmental-1 had been described as artificial so it is believed that the difference in smell of Chips 23 and Chips 27 is that the Chips 23 had been stored for two days more, and more of the unpleasant, artificial smell had vanished. From this test it can be concluded that chips with Emmentel-1 have a pleasant aroma when they reach the consumers. In the future it is also recommended that chips are evaluated after minimum three days from production. Today the minimum time is one day at Santa Maria AB.

The points for the taste of the Chips 27 in the hedonic evaluation, figure 2, did not significantly ($\alpha = 0.05$) differed from the Original chips, and both the median and maximum value were the same for Chips 27 as for the Original chips. According to the comments the panelists gave, the taste profile of all three samples was similar. Hence the Chips 27, which do not contain any synthetic flavorings, matches the objectives for the project since it keeps similar taste profile as the Original, scores same or higher in internal sensory panel and the product cost is only 0.16 % to 0.53 % higher (depending on how the flavorings are bought), which is within the limit of a maximum 10 % increase.

For the declaration of the Tortilla cheese chips, the major change is from ‘flavorings’ to ‘natural flavorings’. The other change in the declaration is the order of the ingredients since some synthetic flavorings were replaced with other ingredients and not only with NA flavorings.

For the Cheese dip mix, the Dip 17 showed the same minimum, median and maximum value as the Original in the first hedonic evaluation of the aroma/taste, figure 6. In the second evaluation the minimum value was higher for the Dip 17, the maximum value was the same and the median was one point lower, compared to the Original dip. No significant ($\alpha = 0.05$) difference were found between the Original and Dip 17. For Dip 18, the point for aroma/taste appears to be lower than the Original, since the first quintile starts at four and a half for Dip 18, and at six for the Original. There is no significant ($\alpha = 0.05$) difference between the two but an indication that the Dip 18 is slightly not as good as the Original. This may be due to several reasons, the aroma/taste may simply not be as pleasant of Dip 18 as of the Original or it may be because that the seasoning mixture for Dip 18 was made only a few hours before the test, and had not been prepared for minimum 24 h as Santa Maria’s recommendations. Due to limited time for the project, a new hedonic evaluation of Dip 18 was not performed. But I would recommend Santa Maria to evaluate Dip 18 one more time and at that occasion make the seasoning mixture minimum a day prior analysis.

Hence, according to the results, Dip 17, which do not contain any synthetic flavorings, is seen as end product of the product development of the Cheese dip mix. The product cost of Dip 17 is 0.45 % higher than the Original dip and as discussed above the aroma/taste scores the same as the Original dip. The R&D specialists taste profile is also similar as the Original, according to R&D specialists at Santa Maria AB. Hence Dip 17 meets the objective of the project.

For the declaration of Dip 17, two different alternatives are available, presented as 1 and 2 in the results. The difference between these two is if the flavorings are declared as ‘natural flavorings’ or as ‘natural jalapeño flavoring with other natural flavorings’. Which one to choose is up to a regulatory specialist together with the product developer to speculate what the consumers would prefer, and what that is possible to make room for on the package.

5.2 Shelf life

Two different methods were tested. The first one, Method 1, was performed on Original chips. The other one, Method 2, on Original chips, Original spice mixture, Chips 14 chips and Chips 14 spice mixture. In the second method it was apprehended that the rancidness of the corn chips would take over so much so differences in the spice mixture would not be noticed, that is why both the spice mixture and the chips was tested. The same would have been done for Method 1 if it would have given good results after the first evaluation.

In figure 11 the results from the first and only evaluation of Method 1 can be found. Two samples stored in the highest temperature used, 42°C, were evaluated, one stored in heat for one week and in -20°C for one and a half week and the other sample stored in heat in two weeks and in -20°C for a half week. The second sample had almost the same sensory profile as the fresh sample, only the point for the parameter ‘Salt’ had decreased one step. The first sample was more changed, the points for four of the parameters had decreased one step. The parameters changed were ‘Cheddar-cheese’, ‘Salt’, ‘Sourness’ and ‘Body’. To evaluate if the freezing step affected the samples a sample stored in -20°C for two and a half week was evaluated. This sample had lower points for three parameters; ‘Salt’, ‘Sourness’ and ‘Body’, one step lower for all of them compared to fresh sample. From these results the conclusion could be drawn that the time period in the freezer affected the samples more than the time period in the heat. It should be pointed out that the zero sample (chips and spice mixture stored in -20°C separately for two and a half week then the chips were heated and the spice mixture was added) had the same sensory profile as fresh chips. So it seems like the heating of the chips in oven affects the taste to go back as it was before freezing, or that the taste change seen in the seasoned chips stored in freezer does not happen in unseasoned chips. Because the bad results Method 1 was rejected as a method possible to use for evaluation of shelf life of seasoned tortilla chips.

For the second method the freezing step was not included and the temperatures were slightly changed to be a bit higher. For the seasoning mixture the temperature highly affected the sample appearance. The higher temperature used, the darker, dryer and clumpier samples, figure 12. This can also be seen in the difference in weight of the samples, before and after storage, figure 21. For the 30°C samples the weight was almost unchanged after the storage, the small change seen is within the margin of error. For both 42°C and 50°C the samples lost weight during the storage. This weight loss may be due to that the spice mixture lost moisture and got burnt during the storage. Also the taste of the spice mixture was described as burnt in some cases. For Chips 14 spice mixture parameter ‘Rancidness’, figure 20, it can be seen that the rancid taste of the spice mixture dropped after 27 days. At this point the spice mixture tasted more burnt and not rancid. All together, the appearance change, the weight loss and the lost rancid taste, is pointing to that 50°C was a too high temperature to use for a spice mixture, it burnt the sample too much.

The availability of air may affect the shelf life since many degradation reactions are dependent on oxygen. To evaluate this, the same amount of spice mixture was stored in two different sizes of bags for 50 days in 42°C. The appearance and texture of the big bag sample was more like the fresh sample than the small bag sample was, figure 13. The sensory profile was also different. The main difference was the smell, the Big bag samples had a much lower intensity of ‘Burnt cauliflower’. This may be due to that the aroma had a bigger volume of air to be dispread into. Overall it can be concluded that the availability of air do affects the spice mixture.

For the smell of the spice mixtures, it was mainly the parameters ‘Off smell’ and ‘Burnt cauliflower smell’ that changed the most. The last smell parameter ‘Rancid smell’ did not change during the storage. The ‘Off smell’ started at point one for the Original spice mixture and on four for Chips 14 spice mixture. After the whole storage period the points were almost the same for both the Original spice mixture and for the Chips 14 spice mixture, for both 42°C and 50°C the points were two for both Original and Chips 14. For 30°C the points were one for Original and three for Chips 14. For the ‘Burnt cauliflower’ parameter both the Original spice mixture and Chips 14 spice mixture followed the same curve, started at two points and ended at four to five for both 42°C and 50°C and at a three for 30°C. From this it can be concluded that the smell formed during storage is burnt/cooked cauliflower and that some ‘Off smell’ can be sensed in some samples.

For the taste, two parameters were affected the most during the storage; ‘Rancidness’ and ‘Cheddar-cheese’. The ‘Cheddar-cheese’ decreased at a higher rate with increased temperature for both the Original and Chips 14 spice mixtures, figure 17 and figure 19. For the Chips 14 spice mixture, the ‘Cheddar-cheese’ decreased at a higher rate with increased temperature compared to the Original spice mixture, and slower at low temperature. Hence Chips 14 spice mixture is more sensitive for high heat and it is possible to speed up reactions corresponding to decreasing ‘Cheddar-cheese’ more for this sample. One week in 42°C corresponds to four weeks in room temperature for Chips 14 spice mixture and only one week and five days for the Original spice mixture. At 30°C the difference in decreasing ‘Cheddar-cheese’ is not as big between the two samples, one week in 30°C corresponds to nine days in room temperature for Chips 14 spice mixture and eight days in room temperature for Original spice mixture. For the other taste parameter, ‘Rancidness’ for the Chips 14 spice mixture it increased up to a four after 27 days and then it dropped down to a one after 50 days. This may be due to that the rancid molecules formed are broken down after a certain time.

For the chips the points for ‘Rancid smell’ was slightly higher for the Original whilst the ‘Off smell’ was higher for ‘Chips 14’. Both these parameters are unwanted in the product, and the results should only be seen as that the smell after storage of the Original chips and Chips 14 are slightly different from each other. For the taste the same parameters as for the spice mixture changed the most; ‘Cheddar-cheese' and 'Rancidness'. The cheese taste, measured by the parameter 'Cheddar-cheese', disappear during the storage and the rancid taste, 'Rancidness', is obtained and increases during storage. The loss of cheese taste is a bit faster for Chips 14 at 42°C and 50°C, but at 30°C it is faster for the Original chips. Since the loss of cheese taste does not give the chips any unpleasant taste the parameter is not affecting the shelf life of the product in any greater extend. The parameter 'Rancidness' on the other hand, does highly affect the shelf life since it is an unwanted taste in the chips.

For chips that has passed the expiring date the 'Rancidness' was given the point four. To calculate what shelf life the products got from Method 2 the data was extrapolated to a point four in 'Rancidness' and the corresponding time in room temperature (295 K) was calculated. When all points were taken into account, the Original chips had a shelf life of five and a half months and the Chips 14 had a shelf life of seven months. The obtained result for Chips 14 was when the last point for 30°C was corrected. It was given one in the evaluation but the sample evaluated two weeks earlier got a two, it is not likely that the 'Rancidness' goes down so fast after the rancidness molecules are formed. It is possible that the rancid molecules started to be break down, but more likely is that the panel missed a rancid note, that is why the correction was done. For the Original chips parameter 'Rancidness' one week in 50°C corresponds to three weeks in room temperature, and for the Chips 14 one week in 50°C corresponds to over five weeks in room temperature. The result for the Original chips is short compared to the shelf life of the product today, which is nine months. The reason for this difference can be explained with that the shelf life of Tortilla cheese chips is highly affected by both temperature and moisture. It is a sensitive product according to the manufacturer, and the real shelf life of the product can vary.

It is possible to calculate the shelf life by only looking at the first samples. This was done for the values up to 27 days after the shelf life study started. By using these data the Original had a shelf life of almost three and a half months whilst Chips 14 had a shelf life of almost five months. The obtained shelf life of the Original is shorter when using the values up to 27 days from start, compared to when all samples were used, and the obtained value of the shelf life is much lower compared to real time measurements. Another way to do the calculations is to start the calculation of reaction rate constants when the reaction starts and end when the reaction seems to end. This was done for both spice mixtures and chips on the parameter 'Rancidness'. One limitation with this method is that the result is highly dependent on which points that are included. For example, if one only takes the last point the parameter score one, and stops at the first point the score is a two, then the result is only dependent on how often samples are analyzed. In this project the calculations were done by using more 'one' points for lower temperature compared to higher since the reaction constant is apprehended to be lower for lower temperature. By using this method the values when the rancidness process has stopped and the molecules formed are broken down, can for example be seen in the 50°C samples for the Chips 14 spice mixture, figure 22, can be excluded from the calculation. Hence the method is good to use in some cases, but in some, the chips in this case, it is not as good as using all point. For the chips the obtained shelf life at the different temperatures varied a lot, from five and a half month to almost ten months for the Original chips, table 20. This is an indication that the calculation is not optimal.

For the chips the samples gained weight during the storage. The lower the temperature, the more increasing weight. This may be due to that some moisture is absorbed and that this moisture is evaporated in higher extend at higher temperature.

The activation energy calculated for 'Cheddar-cheese' and 'Rancidness' for both the spice mixtures and the chips was between zero and twenty. Some had a negative values when using all points to calculate the activation energy, in these cases the activation energy should be calculated in another way. Since the parameter 'Cheddar-cheese' not will be recommended to use in future studies this was only done for 'Rancidness'. The parameter affecting the shelf life the most, 'Rancidness' for the chips had an activation energy of 8 kcal/mole for the Original and 15 kcal/mole for Chips 14 (when using all points). This indicates that the rancidity is due to fat hydrolysis or oxidation since activation energy between 10-

20 kcal/mole indicates a simple hydrolysis reaction and activation energy between 15-250 kcal/mole indicates lipid oxidation.

The results from the shelf life study were highly affected by the expert panel used. Since the panel leader only was trained for a few weeks before she started leading the panel, the results from the study is probably more reliable at the end of the study since she gained more experience during the test. This conclusion is drawn since the method used, consensus profiling, is known to be highly affected by the experience of the panel leader (42). The highly sensitivity of the method may also be seen for the shelf life calculation of Chips 14, if one point is set as a two instead of one (a two means that the attribute is sensed if the panelist try hard to perceive it) the shelf life goes from over fifty months down to seven.

6 Conclusion

It is possible to keep the same or better taste in the Santa Maria products Tortilla cheese chips and Cheese dip mix without using synthetic flavorings and without increasing the product price too much (too much = 10 %). To take away synthetic cheese flavorings it is necessary to replace them with combinations of NA cheese flavorings and body giving components such as cheese powders, milk powder and yeast extracts.

The study gave an indication of that with NA cheese flavorings the Tortilla cheese chips got a longer shelf life than the chips with synthetic flavorings. A shelf life study of the final product, Chips 27, should be conducted since the new cheese powder and the lack of jalapeño flavoring can affect the shelf life. The study also showed that chips with NA flavorings were more sensitive to high heat (42°C and 50°C) than the Original chips are. I would recommend product developer for seasoned tortilla chips to do accelerated shelf life studies on only chips (the taste difference between different spice mixtures were noticeable so no need to test the spice mixture separately) with three different test temperatures between 30°C and 45°C, follow the products for over 50 days and test the products at least at the beginning, in the middle and at the end, but preferably at more points. It is only necessary to evaluate the parameter 'Rancidness'.

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Appendix I

pH value of the original spice mixture was measured by the laboratory at Santa Maria AB with three replicates. The results, as well as the mean value, can be found in table 21.

Table 21. pH value of the spice mixture, three replicates.

Replicate	pH-value
1	4.91
2	4.88
3	4.91
Mean value	**4.90**

Appendix II

Results from the triangle test on Original chips and the Chips 14s can be found in table 22. The panelists were asked to look, smell and taste the chips and find the different one, out of three. An example of the test protocol used can be seen in figure 36 (six different protocols were used, one for each serving order).

Table 22. Results from the triangle test. A = Chips 14, B = Original chips.

				How big difference		**Mark which sample you perfer**
Answer (right/wrong)		**Black**	**Red**	**No noticeable diff**	**Noticeabel diff.**	
Right	Serving BBA - choosing A	1		1		-
	Serving ABB - choosing A	3		2	1	A (A)
	Serving BAB - choosing A	1			1	B&B (B&B)
	Serving ABA - choosing B					
	Serving BAA - choosing B	2		1	1	B (B)
	Serving AAB - choosing B	1			1	
Wrong	Serving BBA - choosing B		1		1	-
	Serving ABB - choosing B					
	Serving BAB - choosing B		3	2	1	B&A (B)
	Serving ABA - choosing A	1	2	1	2	A&B (B)
	Serving BAA - choosing A					
	Serving AAB - choosing A		2	1	1	A&B
	Total			**8**	**9**	
	Total answering right	8				
	Total answering wrong	9				
	Total participants	17				

To verify if there is a significant difference between the two samples, the total number of answering right is compared to a tabulated value. For 17 panelists, if 10 or more participants answer right there is a significant (α = 0.05) difference between the samples (36). In this case, 8 panelists answered right, hence there is no significant (α = 0.05) difference in appearance, smell or taste between the Original chips and the Chips 14.

TRIANGLE TEST

Name:	Date: 20/3-15

You have three samples, two of which are equal. Look, smell and taste all three. Start with the sample to the left.
Identify which one of the three samples is different from the other two.

Sample code:	537	249	386
Which sample is different?			

Mark with a cross, also how big you think the difference is between the two equal and the discrepant sample.

No noticeable difference	Noticeable difference	Clear difference
[ ]	[ ]	[ ]

If you do notice a difference which sample do you prefer (mark both the equal samples if you prefer them)?

537	249	386
[ ]	[ ]	[ ]

Please write a comment on what difference you notice (if you notice a difference):

Figure 36. Example of test protocol used in the triangle test. 386 = Original chips and 537 and 249 = Chips 14.

Appendix III

An example of the test protocol used in the hedonic evaluation of cheese tortilla chips (Original, Chips 23 and Chips 27) can be seen in figure 37 (six different protocols were used, one for each serving order). The panelists were asked to cut the bag open, smell in the bag, set point for the smell and then taste the sample. Figure 38 shows the three samples. The results are shown in table 23.

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Santa Maria

TEST PROTOCOL - SENSORY EVALUATION

Product:	Tortilla chips Cheese		Date:	2015-04-23	Sign:	
Project no:	1151					
		Aroma (when sniffing in bag)		Taste		Overall acceptability
Sample	Score	Comments	Score	Comments	Score	Comments
729						
217						
351						

Are the three samples very similar in taste?

Yes ☐ No ☐

If No, which two samples are the most similar in taste? ______________________

Scoring	
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Figure 37. Example of test protocol used in the hedonic evaluation. 729 = Original chips and 351 = Chips 23 and 217 = Chips 27.

Figure 38. The three chips bags, served to each panelist.

Table 23. Results from the first hedonic evaluation of the Cheese tortilla chips. A = Original chips, B = Chips 23 and Chips 27.

Serving order	Aroma (when sniffing in bag)			Taste			Over all acceptability				Are the three samples very similar?		If No, which two samples are the most similar
	A	B	C	A	B	C	A	B	C		Yes	No	
ABC	8	5	4	8	7	7	8	7	6		x		B & C
	7	7	7	7	7	7	7	7	7			x	B & C
	9	9	8	8	8	7	8	8	7			x	A & B
ACB	8	7	6	8	8	7	8	7	6			x	A & B
	8	7	5	8	7	5	8	7	5			x	A & B
	7	8	7	7	7	8	6	7	8			x	A & B
BAC	7	8	4	8	7	5	8	7	5			x	A & B
	5	7	6	7	6	6	6	6	6		x		-
	4	7	7	5	6	8	8	8	8				-
	5	7	4	7	8	5	7	8	5			x	A & B
BCA	5	6	4	6	6	7	6	6	7		x		-
	8	7	6	7	8	7	8	8	7		x		-
	2	8	8	7	8	8	6	8	8			x	A & C
CAB	6	3	2	6	3	3	6	3	3			x	B & C
	7	7	7	6	7	8	6	7	8			x	A & B
	4	5	7	4	5	7	4	5	7			x	A & B
	8	8	6	8		8						x	A & B
CBA	5	7	6				6	6	8			x	A & C
	6	5	4	6	3	4	6	4	4			x	A & C
	6	6	5	7	7	7	7	7	7			x	A & B
Mean	6,25	6,70	5,65	6,84	6,56	6,53	6,79	6,63	6,42	Total	4	15	

To check if the data, table 23, was normally distributed the MATLAB function Normpot was used. The code can be seen below.

% Test if the data are normal distributed by using function Normplot

```
% AromA
Aa = [2 4 4 5 5 5 5 6 6 6 7 7 7 7 8 8 8 8 8 9]';
Ba = [3 5 5 5 6 6 7 7 7 7 7 7 7 7 7 8 8 8 8 9]';
Ca = [2 4 4 4 4 4 5 5 6 6 6 6 6 7 7 7 7 7 8 8]';
```

```
Xa = [Aa, Ba, Ca];
 figure (1)
Ha = normplot(Xa)
legend('Original','Chips 23','Chips 27');
 % Taste
 % Exluded data from person only giving point to At and Ct
At = [8 7 8 8 8 7 8 7 5 7 6 7 7 6 6 4 6 7]';
Bt = [7 7 8 8 7 7 7 6 6 8 6 8 8 3 7 5 3 7]';
Ct = [7 7 7 7 5 8 5 6 8 5 7 7 8 3 8 7 4 7]';

Xt = [At, Bt, Ct];

figure (2)
Ht = normplot(Xt)
legend('Original','Chips 23','Chips 27');

% Overall acceptablility
 Ao = [8 7 8 8 8 6 8 6 8 7 6 8 6 6 6 4 6 6 7]';
Bo = [7 7 8 7 7 7 7 6 8 8 6 8 8 3 7 5 6 4 7]';
Co = [6 7 7 6 5 8 5 6 8 5 7 7 8 3 8 7 8 4 7]';
 Xo = [Ao, Bo, Co];
 figure (3)
Ho = normplot(Xo)
legend('Original','Chips 23','Chips 27');
```

Produced graphs can be seen in figure 39-41. Since the curves show linear behavior, it is assumable that the data is normally distributed.

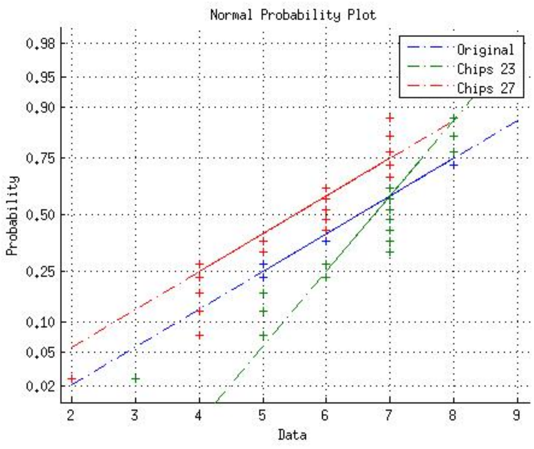


Figure 39. Aroma test

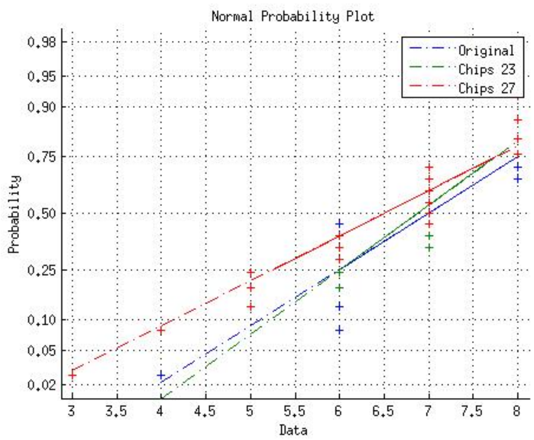


Figure 40. Overall acceptability

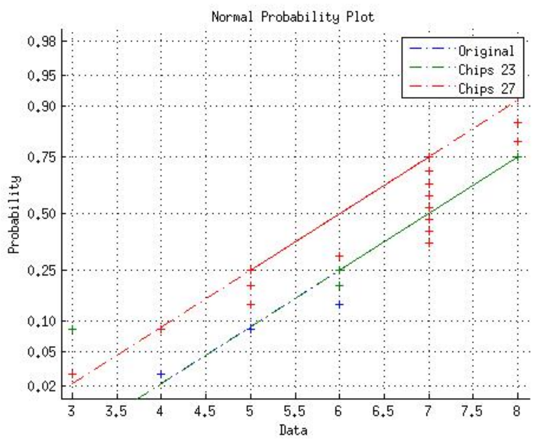


Figure 41. Taste

t-test

Since the data was normally distributed, *t*-test could be used to evaluate the statistical difference between the chips. The Original chips are compared against Chips 23 and Chips 27 and respectively. For the aroma, Chips 23 was also compared to Chips 27. For equations used, see section 2.8.

Aroma Original and Chips 23

Let $\mu_{Original}$ be the true average value of the aroma of the original, and $\mu_{\text{Chips 23}}$ denotes the true average value for the aroma of the Chips 23.

The hypotheses:

H_0: $\mu_{Original} = \mu_{\text{Chips 23}}$ (No difference in the true value of the aroma for the two samples)

H_A: $\mu_{Original} \neq \mu_{\text{Chips 23}}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 6.25$$
$$\bar{\bar{y}} = 6.7$$
$$s_1^2 = 3.14$$
$$s_2^2 = 1.91$$
$$t_{obs} = -0.896$$
$$\nu = 35$$
$$t_{0.025,35} = \pm 2.03$$

Since $-t_{0.025,35} < t_{obs} < +t_{0.025,35}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the aroma between the original and Chips 23.

Taste Original and Chips 23

Let $\mu_{Original}$ be the true average value of the taste of the original, and $\mu_{\text{Chips 23}}$ denotes the true average value for the taste of the Chips 23.

The hypotheses:

H_0: $\mu_{Original} = \mu_{\text{Chips 23}}$ (No difference in the true value of the taste for the two samples)

H_A: $\mu_{Original} \neq \mu_{\text{Chips 23}}$ (Difference between the true values of the taste for the two samples)

$$\bar{x} = 6.84$$
$$\bar{\bar{y}} = 6.56$$
$$s_1^2 = 1.25$$
$$s_2^2 = 2.38$$
$$t_{obs} = 0.64$$
$$\nu = 30$$
$$t_{0.025,30} = \pm 2.04$$

Since $-t_{0.025,30} < t_{obs} < +t_{0.025,30}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the taste between the original and Chips 23.

Overall acceptability Original and Chips 23

Let $\mu_{Original}$ be the true average value of the overall acceptability of the original, and $\mu_{\text{Chips 23}}$ denotes the true average value for the overall acceptability of the Chips 23.

The hypotheses:

H_0: $\mu_{Original} = \mu_{\text{Chips 23}}$ (No difference in the true value of the overall acceptability for the two samples)

H_A: $\mu_{Original} \neq \mu_{\text{Chips 23}}$ (Difference between the true values of the overall acceptability for the two samples)

$$\bar{x} = 6.79$$
$$\bar{\bar{y}} = 6.63$$
$$s_1^2 = 1.29$$
$$s_2^2 = 1.91$$
$$t_{obs} = 0.38$$
$$\nu = 34$$
$$t_{0.025,34} = \pm 2.03$$

Since $-t_{0.025,34} < t_{obs} < +t_{0.025,34}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the overall acceptability between the original and Chips 23.

Aroma Original and Chips 27

Let $\mu_{Original}$ be the true average value of the aroma of the original, and $\mu_{Chips\ 27}$ denotes the true average value for the aroma of the Chips 27.

The hypotheses:

H_0: $\mu_{Original} = \mu_{Chips\ 27}$ (No difference in the true value of the aroma for the two samples)

H_A: $\mu_{Original} \neq \mu_{Chips\ 27}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 6.25$$
$$\bar{\bar{y}} = 5.65$$
$$s_1^2 = 3.14$$
$$s_2^2 = 2.56$$
$$t_{obs} = 1.12$$
$$\nu = 37$$
$$t_{0.025,37} = \pm 2.04$$

Since $-t_{0.025,37} < t_{obs} < +t_{0.025,37}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the aroma between the original and Chips 27.

Taste Original and Chips 27

Let $\mu_{Original}$ be the true average value of the taste of the original, and $\mu_{Chips\ 27}$ denotes the true average value for the taste of the Chips 27.

The hypotheses:

H_0: $\mu_{Original} = \mu_{Chips\ 27}$ (No difference in the true value of the taste for the two samples)

H_A: $\mu_{Original} \neq \mu_{Chips\ 27}$ (Difference between the true values of the taste for the two samples)

$$\bar{x} = 6.84$$
$$\bar{\bar{y}} = 6.53$$
$$s_1^2 = 1.25$$
$$s_2^2 = 2.15$$
$$t_{obs} = 0.75$$
$$\nu = 33$$
$$t_{0.025,33} = \pm 2.03$$

Since $-t_{0.025,33} < t_{obs} < +t_{0.025,33}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the taste between the original and Chips 27.

Overall acceptability Original and Chips 27

Let $\mu_{Original}$ be the true average value of the overall acceptability of the original, and $\mu_{Chips\ 27}$ denotes the true average value for the overall acceptability of the Chips 27.

The hypotheses:

H_0: $\mu_{Original} = \mu_{Chips\ 27}$ (No difference in the true value of the overall acceptability for the two samples)

H_A: $\mu_{Original} \neq \mu_{Chips\ 27}$ (Difference between the true values of the overall acceptability for the two samples)

$$\bar{x} = 6.79$$
$$\bar{\bar{y}} = 6.42$$
$$s_1^2 = 1.29$$
$$s_2^2 = 2.15$$
$$t_{obs} = 0.87$$
$$\nu = 33$$
$$t_{0.025,33} = \pm 2.03$$

Since $-t_{0.025,33} < t_{obs} < +t_{0.025,33}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the overall acceptability between the original and Chips 27.

Aroma Chips 23 and Chips 27

Let $\mu_{Chips\ 23}$ be the true average value of the aroma of the Chips 23, and $\mu_{\text{Chips 27}}$ denotes the true average value for the aroma of the Chips 27.

The hypotheses:

H_0: $\mu_{Chips\ 23} = \mu_{\text{Chips 27}}$ (No difference in the true value of the aroma for the two samples)

H_A: $\mu_{Chips\ 23} \neq \mu_{\text{Chips 27}}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 6.70$$
$$\bar{\bar{y}} = 5.65$$
$$s_1^2 = 1.91$$
$$s_2^2 = 2.56$$
$$t_{obs} = 2.22$$
$$\nu = 37$$
$$t_{0.025,37} = \pm 2.04$$

Since $t_{obs} > t_{0.025,37}$ the noll hypothesis can be rejected, hence there is a significant ($\alpha = 0.05$) difference in point for the aroma between the Chips 23 and Chips 27.

Appendix IV

An example of the test protocol used in the hedonic evaluation of Cheese dip mix (Original, Dip 13 and Dip 17) can be seen in figure 42 (two different protocols were used, one for each serving order). The panelists were asked to first taste the dip with spoon, then together with unseasoned tortilla chips and cucumber. The results are shown in table 24.

Document name: Version: 1 valid from: 2012-08-x: Approved by: Peter Blomgren

Santa Maria

TEST PROTOCOL - SENSORY EVALUATION

Product: Cheese dip mix **Date:** 2015-04-22 **Sign:**

Project no: 1151

	APPERANCE		AROMA/TASTE		TEXTURE	
Sample	**Score**	**Comments**	**Score**	**Comments**	**Score**	**Comments**
571						
382						
937						

Scoring

9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Figure 42. Example of test protocol used in the hedonic evaluation. 571 = Original, 382 = Dip 13 and 937 = Dip 17.

Table 24. Results from the first hedonic evaluation of the cheese dip mix.

Serving order	Apperance Original	Apperance Dip 13	Apperance Dip 17	Aroma/taste Original	Aroma/taste Dip 13	Aroma/taste Dip 17	Texture Original	Texture Dip 13	Texture Dip 17
Original, Dip 13, Dip 17	8	8	8	8	6	6	8	8	8
	7	7	7	7	6	6	7	7	7
	7	7	7	7	8	7	7	7	7
	8	8	8	7	4	8	8	8	8
	7	7	7	6	7	7	7	8	7
Dip 13, Original, Dip 17	7	7	7	7	7	7	7	6	6
	6	6	6	6	7	7	7	7	7
	8	8	8	8	8	8	7	6	6
	8	8	8	8	5	7	7	7	7
	7	7	7	8	6	7	7	7	7
Mean	**7,3**	**7,3**	**7,3**	**7,2**	**6,4**	**7**	**7,2**	**7,1**	**7**

To check if the data, table 24, was normally distributed the MATLAB function Normpot was used. The code can be seen below.

```
% Test if the data are normaldistributed by using function normplot


% Apperance

Aa = [8 7 7 8 7 7 6 8 8 7]';
Ba = [8 7 7 8 7 7 6 8 8 7]';
Ca = [8 7 7 8 7 7 6 8 8 7]';

Xa = [Aa, Ba, Ca];


figure (1)
Ha = normplot(Xa)
legend('Original','Dip 13','Dip 17');

% Aroma/taste

Aat = [8 7 7 7 6 7 6 8 8 8]';
Bat = [6 6 8 4 7 7 7 8 5 6]';
Cat = [6 6 7 8 7 7 7 8 7 7]';

Xt = [Aat, Bat, Cat];

figure (2)
Ht = normplot(Xt)
legend('Original','Dip 13','Dip 17');

% Texture

At = [8 7 7 8 7 7 7 7 7 7]';
Bt = [8 7 7 8 8 6 7 6 7 7]';
Ct = [8 7 7 8 7 6 7 6 7 7]';

Xo = [At, Bt, Ct];

figure (3)
Ho = normplot(Xo)
legend('Original','Dip 13','Dip 17');
```

Produced graphs can be seen in figure 43-45. Since the curves show linear behavior, it is assumable that the data is normally distributed.

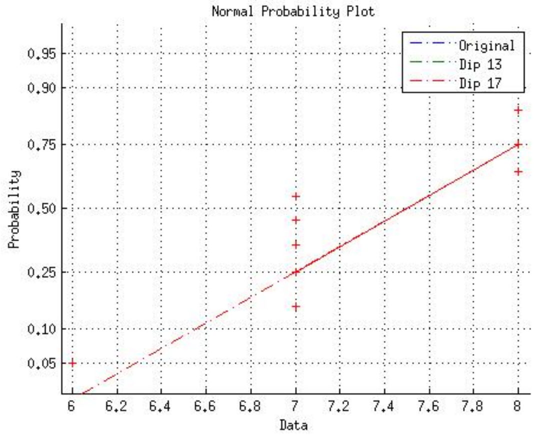


Figure 43. Appearance.

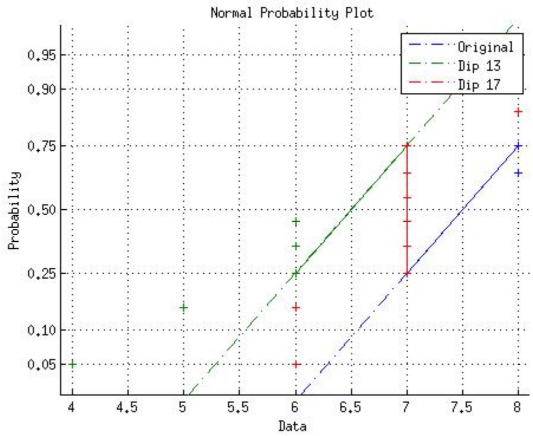


Figure 44. Aroma/taste.

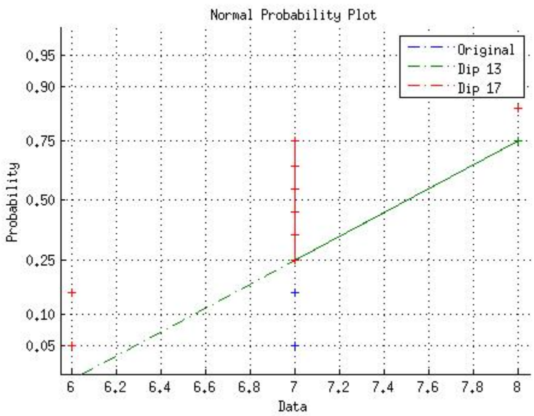


Figure 45. Texture.

t-test

Since the data was normally distributed, t-test could be used to evaluate the statistical difference between the different dips. The Original cheese dip mix is compared against Dip 13 and Dip 17 respectively. For equations used, see section 2.8.

Appearance original and Dip 13

Let $\mu_{Original}$ be the true average value of the appearance of the Original, and $\mu_{Dip\ 13}$ denotes the true average value for the appearance of the Dip 13.

The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 13}$ (No difference in the true value of the aroma for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 13}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 7.3$$
$$\bar{\bar{y}} = 7.3$$
$$s_1^2 = 0.46$$
$$s_2^2 = 0.46$$
$$t_{obs} = 0$$
$$\nu = 18$$
$$t_{0.025,18} = \pm 2.10$$

Since $-t_{0.025,18} < t_{obs} < +t_{0.025,18}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the appearance between the Original and Dip 13.

Aroma/Taste Original and Dip 13
Let $\mu_{Original}$ be the true average value of the aroma/taste of the Original, and $\mu_{Dip\ 13}$ denotes the true average value for the aroma/taste of the Dip 13
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 13}$ (No difference in the true value of the taste for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 13}$ (Difference between the true values of the taste for the two samples)

$$\bar{x} = 7.20$$
$$\bar{\bar{y}} = 6.40$$
$$s_1^2 = 0.62$$
$$s_2^2 = 1.6$$
$$t_{obs} = 1.70$$
$$\nu = 15$$
$$t_{0.025,15} = \pm 2.13$$

Since $-t_{0.025,15} < t_{obs} < +t_{0.025,15}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the aroma/taste between the Original and Dip 13.

Texture Original and Dip 13
Let $\mu_{Original}$ be the true average value of the texture of the Original, and $\mu_{Dip\ 13}$ denotes the true average value for the texture of the Dip 13.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 13}$ (No difference in the true value of the overall acceptability for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 13}$ (Difference between the true values of the overall acceptability for the two samples)

$$\bar{x} = 7.2$$
$$\bar{\bar{y}} = 7.1$$
$$s_1^2 = 0.18$$
$$s_2^2 = 0.54$$
$$t_{obs} = 0.37$$
$$\nu = 14$$
$$t_{0.025,14} = \pm 2.15$$

Since $-t_{0.025,14} < t_{obs} < +t_{0.025,14}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the texture between the Original and Dip 13.

Appearance Original and Dip 17

Let $\mu_{Original}$ be the true average value of the appearance of the Original, and $\mu_{Dip\ 17}$ denotes the true average value for the appearance of the Dip 17.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 17}$ (No difference in the true value of the aroma for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 17}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 7.3$$
$$\bar{\bar{y}} = 7.3$$
$$s_1^2 = 0.46$$
$$s_2^2 = 0.46$$
$$t_{obs} = 0$$
$$\nu = 18$$
$$t_{0.025,18} = \pm 2.10$$

Since $-t_{0.025,18} < t_{obs} < +t_{0.025,18}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the appearance between the Original and Dip 17.

Aroma/Taste Original and Dip 17
Let $\mu_{Original}$ be the true average value of the aroma/taste of the Original, and $\mu_{Dip\ 17}$ denotes the true average value for the aroma/taste of the Dip 17.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 17}$ (No difference in the true value of the taste for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 17}$ (Difference between the true values of the taste for the two samples)

$$\bar{x} = 7.20$$
$$\bar{\bar{y}} = 7.00$$
$$s_1^2 = 0.62$$
$$s_2^2 = 0.44$$
$$t_{obs} = 0.61$$
$$\nu = 17$$
$$t_{0.025,17} = \pm 2.11$$

Since $-t_{0.025,17} < t_{obs} < +t_{0.025,17}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the aroma/taste between the Original and Dip 17.

Texture Original and Dip 17
Let $\mu_{Original}$ be the true average value of the texture of the Original, and $\mu_{Dip\ 17}$ denotes the true average value for the texture of the Dip 17.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 17}$ (No difference in the true value of the overall acceptability for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 17}$ (Difference between the true values of the overall acceptability for the two samples)

$$\bar{x} = 7.20$$
$$\bar{\bar{y}} = 7.00$$
$$s_1^2 = 0.18$$
$$s_2^2 = 0.44$$
$$t_{obs} = 0.80$$
$$\nu = 15$$
$$t_{0.025,15} = \pm 2.13$$

Since $-t_{0.025,15} < t_{obs} < +t_{0.025,15}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the texture between the Original and Dip 17.

Appendix V

An example of the test protocol used in the hedonic evaluation of Cheese dip mix (Original, Dip 17 and Dip 18) can be seen in figure 46 (six different protocols were used, one for each serving order). The dip was served together with spoons and tortilla chips. Each panelist could choose how to taste the dip. The results are shown in table 25.

Document name: Version: 1 valid from: 2012-08-x: Approved by: Peter Blomgren

TEST PROTOCOL - SENSORY EVALUATION

Product:	**Cheese dip mix**		**Date:**	2015-05-13	**Sign:**	
Project no:	1151					
	APPERANCE		**AROMA/TASTE**		**TEXTURE**	
Sample	**Score**	**Comments**	**Score**	**Comments**	**Score**	**Comments**
832						
397						
751						

Scoring	
9	**Like extremely**
8	**Like very much**
7	**Like moderately**
6	**Like slightly**
5	**Neither like nor dislike**
4	**Dislike slightly**
3	**Dislike moderately**
2	**Dislike very much**
1	**Dislike extremely**

Figure 46. Example of test protocol used in the hedonic evaluation. 751 = Original, 832 = Dip 17 and 397 = Dip 18.

Table 25. Results from the second hedonic evaluation of the cheese dip mix. A = Original, B = Dip 17 and C = Dip 18

	Apperance			Aroma/taste			Texture		
Serving order	A	B	C	A	B	C	A	B	C
ABC	8	8	8	7	7	8	8	8	8
	6	6	6	7	6	6	7	7	7
	7	7	7	7	6	4	7	7	7
ACB	7	7	7	6	7	7	7	7	7
	8	8	8	8	7	4	7	7	7
	7	7	7	8	6	4	8	7	6
BAC	5	5	5	8	7	7	5	5	4
	8	8	8	6	8	4	7	7	7
	8	8	8	4	6	4	6	6	6
BCA	7	8	7	8	6	7	6	6	7
	6	4	7	1	2	6	5	3	8
	7	7	7	8	6	7	7	7	7
CAB	6	6	6	6	7	5	5	5	5
	8	8	7	9	9	8	9	7	8
	8	8	8	6	6	7	7	7	7
CBA	7	7	7	5	6	5	6	6	6
	8	8	8	6	8	7	8	8	8
	8	8	8	8	6	5	8	8	8
Mean value	**7,2**	**7,1**	**7,2**	**6,6**	**6,4**	**5,8**	**6,8**	**6,6**	**6,8**

To check if the data, table 25, was normally distributed the MATLAB function Normpot was used. The code can be seen below.

```
% Test if the data are normaldistributed by using function normplot

% Apperance

Aa = [8 6 7 7 8 7 5 8 8 7 6 7 6 8 8 7 8 8]';
Ba = [8 6 7 7 8 7 5 8 8 8 4 7 6 8 8 7 8 8]';
Ca = [8 6 7 7 8 7 5 8 8 7 7 7 6 7 8 7 8 8]';

Xa = [Aa, Ba, Ca];

figure (1)
Ha = normplot(Xa)
legend('Original','Dip 17','Dip 18');

% Aroma/taste

Aat = [7 7 7 6 8 8 8 6 4 8 1 8 6 9 6 5 6 8]';
Bat = [7 6 6 7 7 6 7 8 6 6 2 6 7 9 6 6 8 6]';
Cat = [8 6 4 7 4 4 7 4 4 7 6 7 5 8 7 5 7 5]';

Xt = [Aat, Bat, Cat];

figure (2)
Ht = normplot(Xt)
```

```
legend('Original','Dip 17','Dip 18');

% Texture

At = [8 7 7 7 7 8 5 7 6 6 5 7 5 9 7 6 8 8]';
Bt = [8 7 7 7 7 7 5 7 6 6 3 7 5 7 7 6 8 8]';
Ct = [8 7 7 7 7 6 4 7 6 7 8 7 5 8 7 6 8 8]';

Xo = [At, Bt, Ct];

figure (3)
Ho = normplot(Xo)
legend('Original','Dip 17','Dip 18');
```

Produced graphs can be seen in figure 47-49. Since the curves show linear behavior, it is assumable that the data is normally distributed.

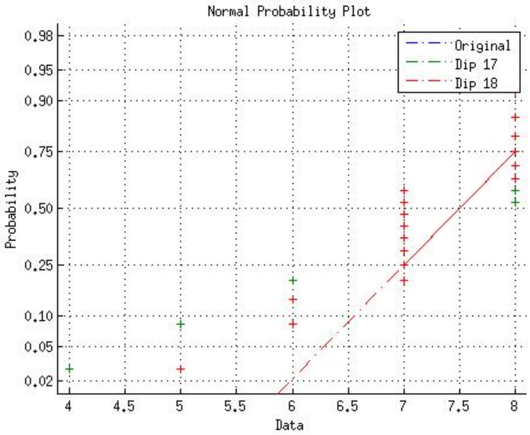


Figure 47. Appearance.

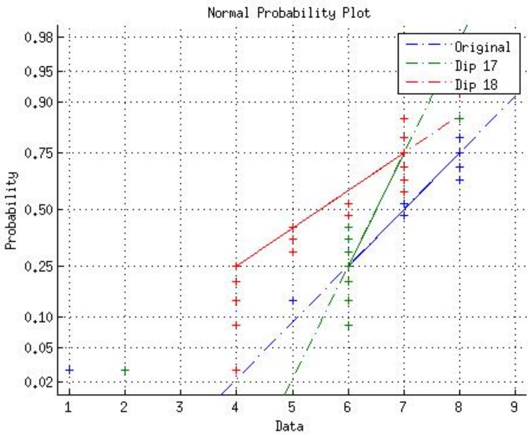


Figure 48. Aroma/taste.

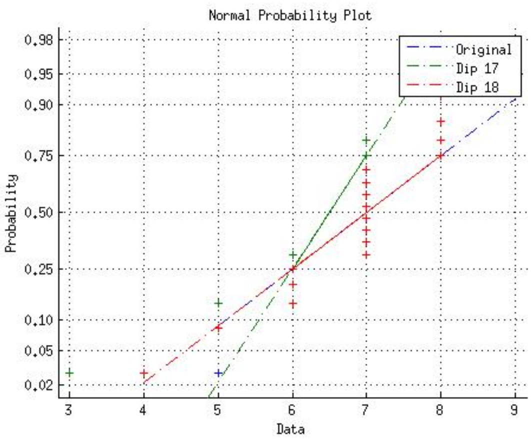


Figure 49. Texture.

t-test

Since the data was normally distributed, *t*-test could be used to evaluate the statistical difference between the different dips. The Original cheese dip mix is compared against Dip 17 and Dip 18 respectively. For equations used, see section 2.8.

Appearance Original and Dip 17

Let $\mu_{Original}$ be the true average value of the appearance of the Original, and $\mu_{Dip\ 17}$ denotes the true

average value for the appearance of the Dip 17.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 17}$ (No difference in the true value of the aroma for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 17}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 7.17$$
$$\bar{\bar{y}} = 7.11$$
$$s_1^2 = 0.85$$
$$s_2^2 = 1.40$$
$$t_{obs} = 0.16$$
$$\nu = 32$$
$$t_{0.025,32} = \pm 2.03$$

Since $-t_{0.025,32} < t_{obs} < +t_{0.025,32}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the appearance between the Original and Dip 17.

Aroma/Taste Original and Dip 17
Let $\mu_{Original}$ be the true average value of the aroma/taste of the Original, and $\mu_{Dip\ 17}$ denotes the true average value for the aroma/taste of the Dip 17.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 17}$ (No difference in the true value of the taste for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 17}$ (Difference between the true values of the taste for the two samples)

$$\bar{x} = 6.56$$
$$\bar{\bar{y}} = 6.44$$
$$s_1^2 = 3.56$$
$$s_2^2 = 2.03$$
$$t_{obs} = 0.20$$
$$\nu = 31$$
$$t_{0.025,31} = \pm 2.02$$

Since $-t_{0.025,31} < t_{obs} < +t_{0.025,31}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the aroma/taste between the Original and Dip 17.

Texture Original and Dip 17
Let $\mu_{Original}$ be the true average value of the texture of the Original, and $\mu_{Dip\ 17}$ denotes the true average value for the texture of the Dip 17.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 17}$ (No difference in the true value of the overall acceptability for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 17}$ (Difference between the true values of the overall acceptability for the two samples)

$$\bar{x} = 6.83$$
$$\bar{\bar{y}} = 6.56$$
$$s_1^2 = 1.32$$
$$s_2^2 = 1.56$$
$$t_{obs} = 0.69$$
$$\nu = 33$$
$$t_{0.025,33} = \pm 2.03$$

Since $-t_{0.025,33} < t_{obs} < +t_{0.025,33}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the texture between the Original and Dip 17.

Appearance original and Dip 18
Let $\mu_{Original}$ be the true average value of the appearance of the Original, and $\mu_{Dip\ 18}$ denotes the true average value for the appearance of the Dip 18.

The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 18}$ (No difference in the true value of the aroma for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 18}$ (Difference between the true values of the aroma for the two samples)

$$\bar{x} = 7.17$$
$$\bar{\bar{y}} = 7.17$$
$$s_1^2 = 0.85$$
$$s_2^2 = 0.74$$
$$t_{obs} = 0$$
$$v = 33$$
$$t_{0.025,33} = \pm 2.03$$

Since $-t_{0.025,33} < t_{obs} < +t_{0.025,33}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the appearance between the Original and Dip 18.

Aroma/Taste Original and Dip 18

Let $\mu_{Original}$ be the true average value of the aroma/taste of the Original, and $\mu_{Dip\ 18}$ denotes the true average value for the aroma/taste of the Dip 18.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 18}$ (No difference in the true value of the taste for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 18}$ (Difference between the true values of the taste for the two samples)

$$\bar{x} = 6.56$$
$$\bar{\bar{y}} = 5.83$$
$$s_1^2 = 3.55$$
$$s_2^2 = 2.15$$
$$t_{obs} = 1.28$$
$$v = 32$$
$$t_{0.025,32} = \pm 2.03$$

Since $-t_{0.025,32} < t_{obs} < +t_{0.025,32}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the aroma/taste between the Original and Dip 18.

Texture Original and Dip 18

Let $\mu_{Original}$ be the true average value of the texture of the Original, and $\mu_{Dip\ 18}$ denotes the true average value for the texture of the Dip 18.
The hypotheses:
H_0: $\mu_{Original} = \mu_{Dip\ 18}$ (No difference in the true value of the overall acceptability for the two samples)
H_A: $\mu_{Original} \neq \mu_{Dip\ 18}$ (Difference between the true values of the overall acceptability for the two samples)

$$\bar{x} = 6.83$$
$$\bar{\bar{y}} = 6.83$$
$$s_1^2 = 1.32$$
$$s_2^2 = 1.21$$
$$t_{obs} = 0$$
$$v = 33$$
$$t_{0.025,33} = \pm 2.03$$

Since $-t_{0.025,33} < t_{obs} < +t_{0.025,33}$ the noll hypothesis cannot be rejected, hence there is no significant (α = 0.05) difference in point for the texture between the Original and Dip 18.

Appendix VI

The results from the training of the expert panel are shown in table 26-29.

Table 26. Words used by the panel to describe the chips and the spice mixture respectively.

Chips 1	Spice mixture (Chips 1)
Salt	Sweet
Cheese	Cheddar
Matured cheddar	Salty
Chili heat	Paprika
Sweet	Onion
Onion	Bitter aftertaste
Paprika	Chili heat weak
Corn	Sourness
Cheese-doodles	Heat
Sweet paprika	
Fried oil	
Puke	
Heat	

Table 27. Results from the cheese tasting.

Cheddar - cheeses		Emmental - cheeses	
Sample	**Results**	**Sample**	**Results**
Swedish (24 month old)	Much flavor	**Cavestored**	Rough Little bit of puke-taste Crumbly, dry, sour, bitter
English	Little sourer than the Swedish. But this has also a lot of taste	**Sliced**	Mild, like Grevé cheese Gummy feeling, a little bit of puke
Sliced	Fatty, weak taste	**Hamburger**	Creamy
Burger cheese	Taste like cheese doodles in the aftertaste Creamy	**Emmental flavor in water (0.1 %)**	Like aquarium Creamy Not so specific cheese Thinks the cheeses are so different so hard to find a cheese that matches the flavor Nutty taste, little bit strange
Cheddar flavor in water (0.1 %)	Weak. Like blue cheese More like the Swedish cheddar or burger cheese Most like the burger cheese In the mouth it feels like the sliced one (fatty feeling) More artificial taste than the Swedish/English	**All emmental cheeses**	So different! “I would not say it was the same type of cheese”
Chips 1	Not like the Swedish cheddar Sour milk taste	**Chips 1**	Thinks the dullness (dovhet) in the chips is emmental. The aftertaste may be the emmental Hard to find a specific cheese-taste Is not a clean cheddar/emmental taste
Spice mixture	Like the Burger cheese		

Table 28. Results from the test with chips/spice mixture changed in a specific way.

	Sample with extra salt (40% extra)	Sample with more sugar (50% extra)	Sample with more cheddar flavor (30% extra)
Panelist 1	Salty, Strong taste	Sweet paprika. No specific cheese taste	More cheesy, more mild, more cheddar
Panelist 2	More intense, more sour, more heat	More sweet, round taste, more maize	More cheddar
Panelist 3	Stronger, 'Stickigare', more sour, less cheese	More cheese, round, sweet	More emmental, Stickig/sharper taste. Less sugar?
Panelist 4	More salt, less cheddar	Milk powder, more creamy, more cheese taste, extra salt	Less salt, cheese, much like ref.

Table 29. Results from the cheese doodles tasting.

Sample name	Appearance	Taste
Cheese doodles the cheezier snack, original (OLW)	Light color. "Typical cheese doodle	More salty than the Cheez starz. Mild. Not as creamy as the Cheez starz
Ostbågar med äkta prästost (Estrella)	Darker than OLW's	More cheesetaste, rich taste. Gouda-cheese taste. Can eat a lot of these. Strong taste, like real cheese.
Ostbågar med äkta cheddar (Estrella)	Darker than OLW's	More cheesetaste. Matured cheese-taste Cheese-salt aftertaste
Cheez starz the cheezier snack, cream cheese & cheddar (OLW)	Light	Creamy, mild, boring

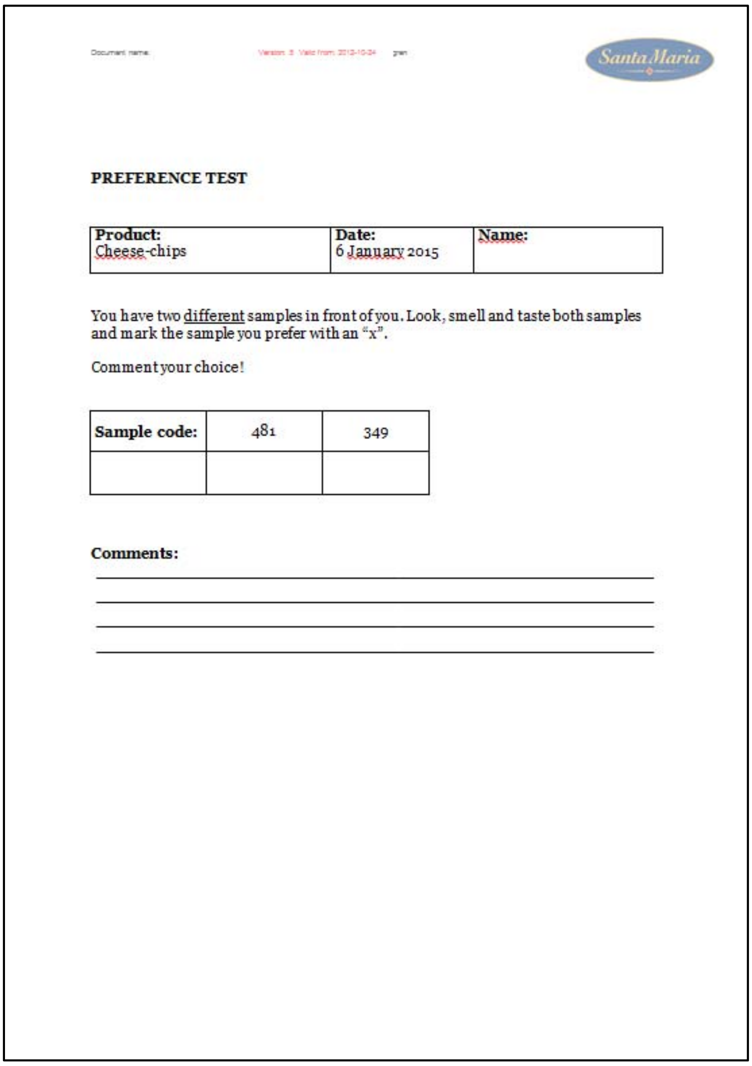
Appendix VII

Results from the paired preference test between the ”Save the raw material” chips and the original cheese tortilla chips can be found in table 30. An example of the test protocol used can be seen in figure 50 (two different protocols were used, one for each serving order).

Table 30. Results from the paired preference test of “Save the raw material” chips (A) and Original chips (B).

Serving order	Save the raw material (A)	Original (B)
AB	1	6
BA	2	5
Total	*3*	*11*

The results were compared to tabulated values (36). With a significance of 5 %, 12 people out of 14 have to choose the same sample out of two, to say that more participants prefer one sample over the other. In this case, 11 chose the same sample, so not a significant results, but an indication of that more participants preferred the original chips over the “save the raw material” chips.



Santa Maria

PREFERENCE TEST

Product: Cheese-chips	**Date:** 6 January 2015	**Name:**

You have two different samples in front of you. Look, smell and taste both samples and mark the sample you prefer with an “x”.

Comment your choice!

Sample code:	481	349

Comments:

Figure 50.Example of test protocol used in the paired preference test. 481 = Save the raw material and 349 = Original.

Appendix VIII

Temperatures in the climate cabinets during Method 1, figure 51, and Method 2, figure 52).

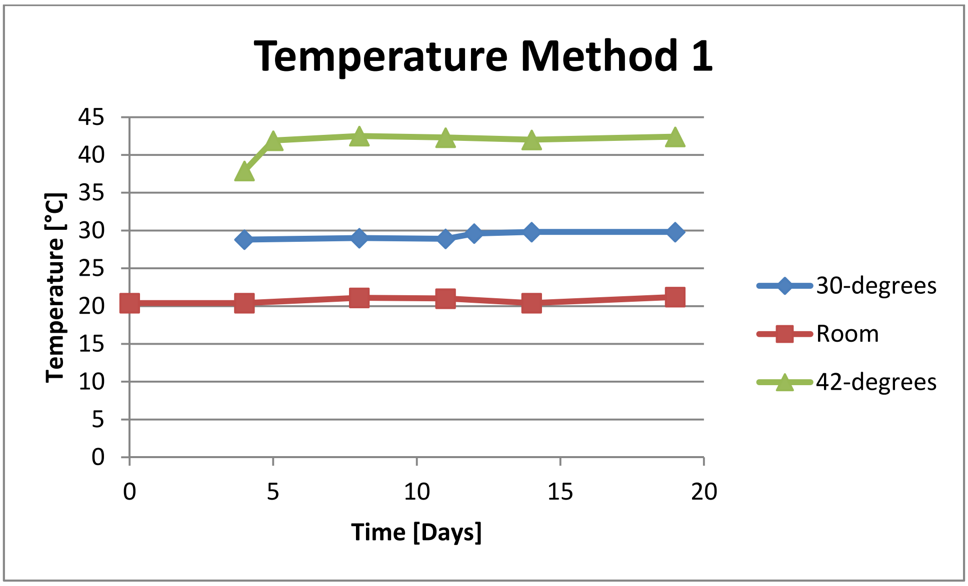


Figure 51. Temperature in the climate cabinets during method 1. The cut-out temperature on the 42°C chamber was to low until day 5 when it was increased. The set temperature on the 30°C chamber was increased after 8 and after 11 days, one degree each time. The time is from the start of Method 1.

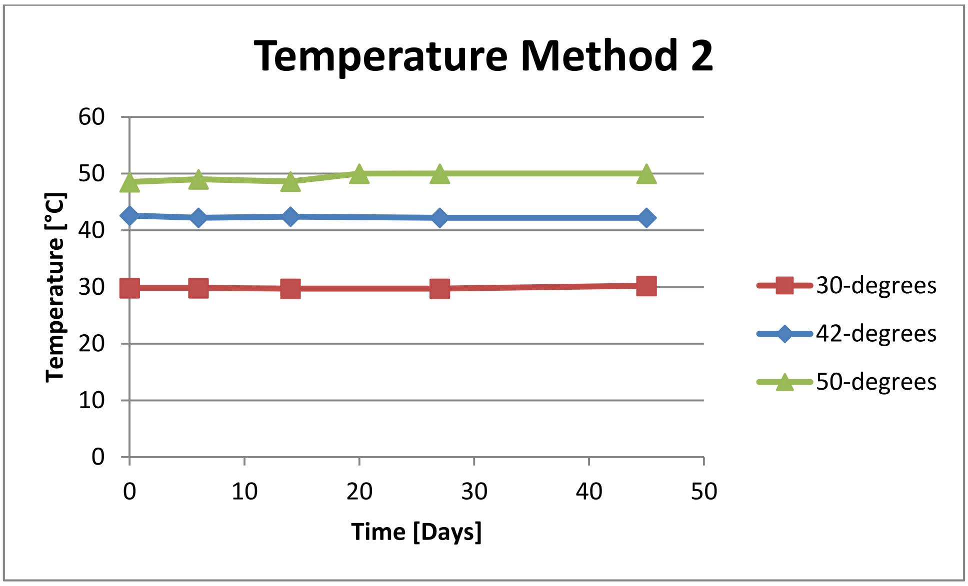


Figure 52. Temperature in the climate cabinets during method 2. The set temperature on the 50°C chamber was increased one degree after 15 days. The time is from the start of Method 2.

Appendix IX

Zero order and first order reaction plots for the Original chips and Chips 14 can be found in figure 53-56. The one with a R^2 value closest to one is the one to choose. Since several of them had the same or more close to one for zero order that was the one used for all parameters. The same results were seen for the spice mixtures, results not shown.

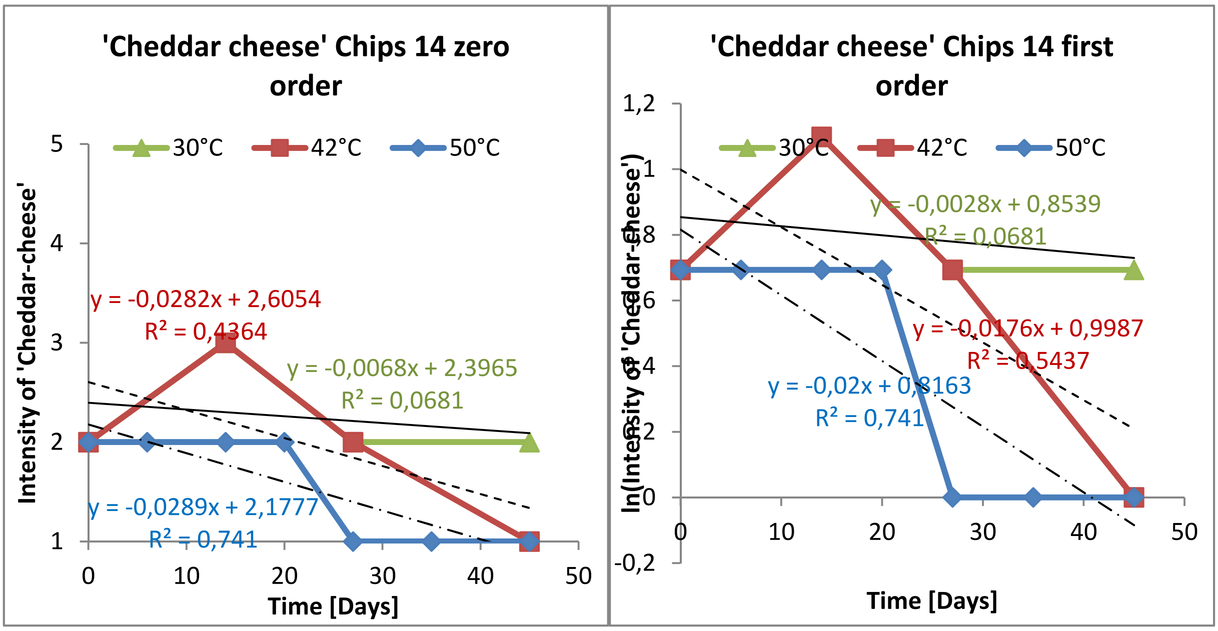


Figure 53. Zero order and first order reaction of 'Cheddar-cheese' for Chips 14.

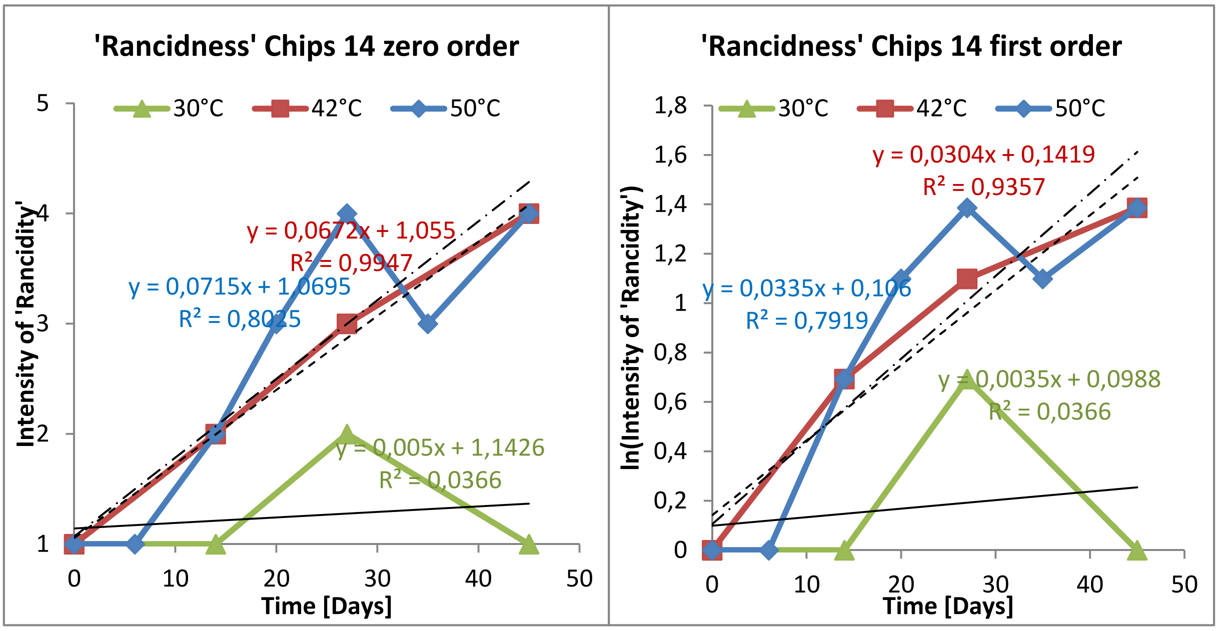


Figure 54. Zero order and first order reaction of 'Rancidness' for Chips 14.

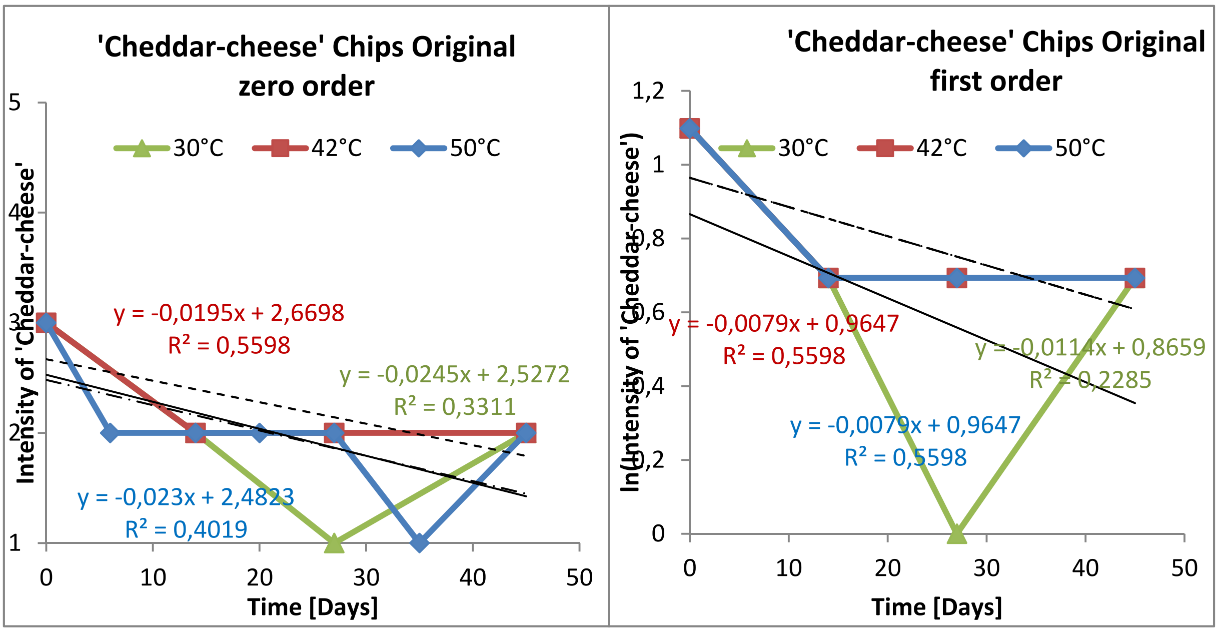


Figure 55. Zero order and first order reaction of 'Cheddar-cheese' for the Original chips.

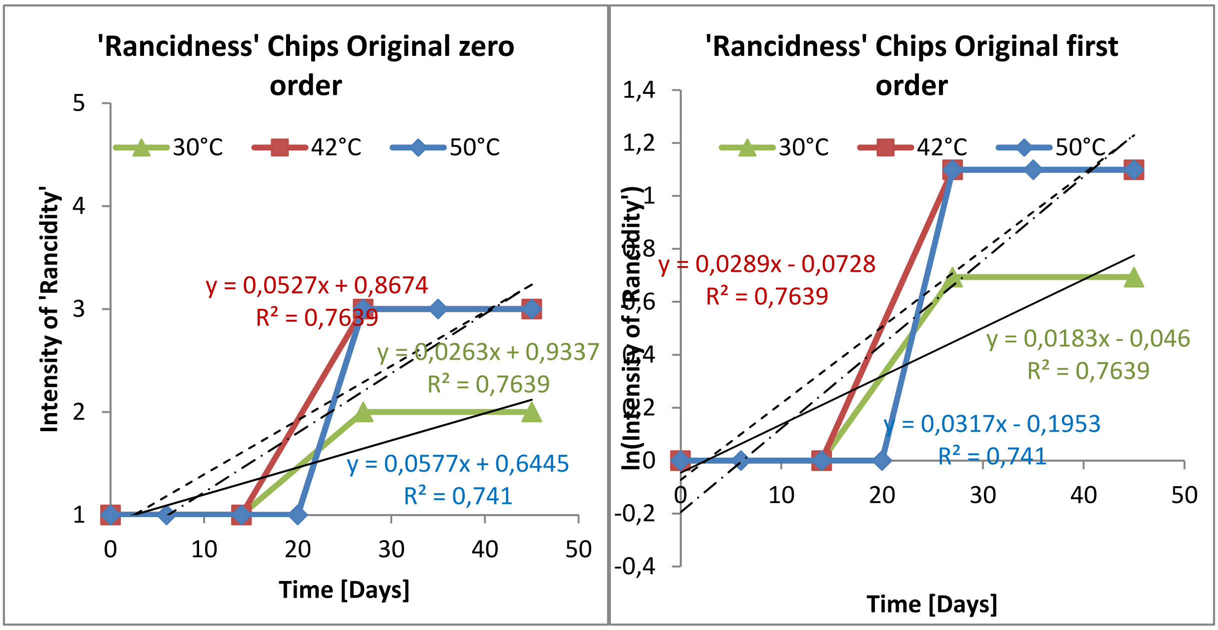


Figure 56. Zero order and first order reaction of 'Rancidness' for the Original chips.

Appendix X

Results from the accelerated shelf life study Method 2 are presented in table 31-34.

Table 31. Results from the screening analysis of Original spice mixture. Pink is 30°C, green is 42°C, blue is 50°C. The sample in bold and italic is the Big bag sample.

Weight before (g)	Weight after (g)	Days old	Results										
			Cheddar cheese	Salt	Chili-heat	Sweetness	Sourness	Rancidness	Body	Paprika	Rancid smell	Off smell	Burnt cauliflower smell
		0	4	5	2	3	4	1	3	2	1	1	2
24,91	24,91	20	4	5	3	3	4	1	3	2	1	2	3
24,81	24,8	35	3	5	2	3	4	1	3	2	1	4	4
24,78	24,79	50	2	5	2	3	4	3	3	2	1	1	3
24,98	24,91	20	2	5	2	3	4	2	2	2	1	1	4
24,88	24,75	35	1	5	2	3	4	3	2	2	1	1	5
24,84	24,63	50	1	5	2	3	4	4	2	2	1	2	5
30,08	***29,69***	***50***	***1***	***5***	***2***	***3***	***4***	***3***	***2***	***2***	***1***	***2***	***2***
24,96	24,93	6	3	5	2	3	4	1	3	2	1	3	4
24,86	24,8	14	2	5	3	3	4	1	2	2	1	3	5
24,91	24,82	20	2	5	3	3	4	1	2	2	1	1	4
24,83	24,66	27	1	5	2	3	3	3	2	2	1	3	5
24,9	24,67	35	1	5	2	2	4	2	2	2	1	2	5
24,82	24,47	50	1	5	2	3	4	3	2	1	1	2	4

Table 32. Results from the screening analysis of Original chips. Pink is 30°C, green is 42°C, blue is 50°C.

Weight before (g)	Weight after (g)	Days old	Results									
			Chedda r-cheese	Salt	Chili -heat	Sweetness	Sourness	Rancidness	Body	Deep-fried-corn	Rancid smell	Off smell
		0	3	4	2	2	3	1	4	3	1	1
52,9	52,9	14	2	4	2	2	3	1	4	3	3	1
52,95	52,99	27	1	4	2	2	2	2	2	3	3	2
53,3	53,38	45	2	4	2	2	2	2	3	2	3	2
52,68	52,68	14	2	4	2	2	3	1	3	3	3	1
52,93	52,96	27	2	4	2	2	2	3	3	3	3	2
53,11	53,19	45	2	4	2	2	2	3	2	2	3	2
52,94												
52,61	52,62	6	2	4	2	2	3	1	3	3	2	2
52,81	52,84	14	2	4	2	2	3	1	4	3	3	1

53,02	53,04	20	2	4	2	2	2	1	3	3	3	1
52,83	52,85	27	2	4	2	2	2	3	2	3	3	2
52,8	52,81	35	1	4	2	2	2	3	3	3	4	1
52,8	52,81	45	2	4	2	2	2	3	3	3	4	2
26 days over expiring date			1	4	2	2	3	4	2	2	4	1

Table 33. Results from the screening analysis of Chips 14 spice mixture. Pink is 30°C, green is 42°C, blue is 50°C. The sample in bold and italic is the Big bag sample.

Bag number	Weight before (g)	Weight after (g)	Days old	**Results**										
				Cheddar cheese	Salt	Chili-heat	Sweetness	Sourness	Rancidness	Body	Paprika	Rancid smell	Off smell	Burnt cauliflower smell
0-sample -1				4	5	2	3	4	1	3	2	1	3	2
0-sample -2				4	5	2	3	4	1	3	2	1	3	2
0-sample			**0**	**4**	**5**	**2**	**3**	**4**	**1**	**3**	**2**	**1**	**4**	**2**
A	25	24,6	20	3	5	3	3	4	2	3	2	1	4	3
B	25	24,8	35	3	5	2	3	4	3	3	2	1	3	3
C	25	24,7	50	3	5	2	3	4	3	3	2	1	3	3
F	25	24,7	20	3	5	3	3	4	1	3	2	1	2	4
G	25	24,6	35	2	5	2	3	4	1	3	2	1	2	4
H	25	24,6	50	1	5	2	3	3	3	2	2	1	2	5
S	***30***	***29,6***	***50***	***1***	***5***	***2***	***3***	***4***	***3***	***2***	***2***	***1***	***1***	***3***
L	25	24,7	6	2	5	2	3	4	1	2	2	1	3	4
M	25	24,6	14	1	5	4	3	4	2	2	3	1	2	4
N	25	24,4	20	1	5	3	3	4	2	2	2	2	2	3
O	25	24,4	27	1	5	2	3	2	4	2	2	1	3	5
P	25	24,5	35	1	5	2	2	3	3	2	2	1	3	5
Q	25	24,6	50	1	5	2	2	4	1	2	2	1	2	5

Table 34. Results from the screening analysis of Chips 14. Pink is 30°C, green is 42°C, blue is 50°C.

Bag number	Weight before (g)	Weight after (g)	Day old	Results									
				Cheddar cheese	Salt	Chili-heat	Sweetness	Sourness	Rancidness	Body	Deep-fried-corn	Rancid smell	Off smell
0-sample-1				2	4	2	2	3	1	3	3	2	3
0-sample-2				2	4	2	2	2	1	3	3	2	2
0-sample-3				2	4	2	2	2	1	3	3	2	3
0-sample-4				2	4	2	2	3	1	4	3	2	3
			0	2	4	2	2	2	1	3	3	2	3
A	52,85	52,89	14	3	4	2	2	3	1	4	3	2	1
B	52,66	52,68	27	2	4	2	2	2	2	3	3	2	3
C	52,91	53,00	45	2	4	2	2	3	1	3	3	2	2
E	53,19	53,23	14	3	4	2	2	2	2	3	3	3	2
F	52,72	52,75	27	2	4	2	2	2	3	2	3	2	3
G	52,55	52,62	45	1	4	2	2	2	4	2	2	4	2
I	53,25	53,26	6	2	4	2	2	2	1	3	2	2	1
J	52,97	53,01	14	2	4	2	2	3	2	3	3	2	2
K	52,5	52,55	20	2	4	2	2	2	3	3	3	3	2
L	53,11	53,14	27	1	4	2	2	2	4	2	2	3	3
M	52,67	52,68	35	1	4	2	2	2	3	2	3	3	2
N	52,52	52,56	45	1	4	2	2	3	4	2	2	3	4