



White Paper

# Importance of Oxidative Stability in the Shelf Life of Foods and Ingredients

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## **Importance of Oxidative Stability in the Shelf Life of Foods and Ingredients**

One of the most important considerations when developing a new product or reformulating an existing one is to ensure that the desired attributes and quality are maintained throughout its entire shelf life. Ingredients, foods and supplements can undergo deteriorative changes during their shelf life that can impact on their chemical, sensory and nutritional properties (texture, appearance, flavour, nutritional value, beneficial health effects). The factors that can cause these changes are varied and include moisture loss/gain, fat degradation or migration, alterations in colour and reactions such as hydrolysis and oxidation that impact flavour compounds.

Often products are considered unacceptable and rejected by consumers due to changes in flavour, with one of the most pronounced effects being the generation of rancid off-flavours/notes caused by the oxidation of oils and fats (and other food components). This chemical decomposition can result in the product being unpalatable. It must also be remembered however, that some of these flavours caused by degradation of fats can be desirable in products such as aged cheese.

Oxidation of fats or oils is a complex process initiated by free radical reactions at the double bonds of unsaturated fatty acids. Therefore, the greater the number of double bonds or degree of unsaturation of the fatty acids, the greater the susceptibility to oxidation. The process of oxidation is affected by many factors including atmospheric oxygen, heat, heavy metals, exposure to light, and other chemical components that promote initiation of the oxidation process. These factors can promote the formation of free radicals which lead to the formation of peroxide radicals, hydroperoxides and subsequent chain reactions leading to the formation of secondary oxidation products including aldehydes and ketones. It is these secondary oxidation products that often produce the distinctive and generally undesirable rancid off flavours/notes and the accumulation of these components over time increases the likelihood of the product being rejected.

As mentioned above, some oils and fats are more prone to oxidation than others such as those high in unsaturated fatty acids, especially polyunsaturated which includes omega 3 and 6 fatty acids. Some of these oils and fats will have natural levels of antioxidants that can counteract the process of oxidation to a certain degree; however the protective effective will eventually be exhausted. This often coincides with an increase in oxidation of the unsaturated fatty acids and generation of rancidity.

### **Reformulation and Processing Options**

There is of course the option for product developers to use more stable fats with less unsaturated and polyunsaturated fatty acids. However, with the trend in

recent times to reduce the level of saturated fat, which is generally more stable, and increase the level of unsaturated/polyunsaturated fatty acids and/or fortify with omega 3 fatty acids for health benefits, there is the challenge to produce formulations that can last the desired/required shelf life.

Product developers might reformulate the fat used in a product to reduce the level of saturated fatty acid by blending with more unsaturated liquid oil. Whilst this would reduce the level of saturated fatty acids, it would also affect the stability of the product. Thereby requiring assessments to be made to optimise the blends to ensure that product integrity could be maintained throughout shelf life.

Changes in formulation of product might also require changes in processing conditions and these can have an impact on the long term oxidative stability of the product. Optimising process conditions can also lead to extension of shelf life of the product which may result in less wastage, greater flexibility and improved profitability of a product. Manufacturing processes can be very complex with multiple stages and many possible opportunities to improve oxidative stability of the finished product, for example, by reducing temperatures, residence time, aeration and type of aeration gas. It can often be beneficial to assess the impact of these different conditions and optimise to improve stability.

One tool that can be used to improve the stability of reformulated products that have more unsaturated fatty acids or have higher levels of (added) polyunsaturated fatty acids such as omega 3 is the addition of antioxidants. As previously mentioned, these can delay the onset of oxidation and generation of rancidity. The selection of antioxidant and optimisation of levels requires assessment of the effectiveness in preventing/delaying oxidation. This potentially involves assessing many different permutations of antioxidant and levels to be added.

### **Oxidative Stability/Shelf Life Assessment Techniques Available**

As discussed, it is important to ensure that a product is stable and with reference to this paper, resistant to oxidation under the conditions it will be exposed to over the period of its shelf life. This can be a very time-consuming process particularly if products have long shelf lives and can impact the time of product to market.

Accelerated studies can be conducted to speed up this process in which the product is exposed to harsher conditions such as higher temperature, aeration and exposure to UV light or trace metals. Typically however

elevated temperature and aeration are used. It is however imperative to ensure that this accelerated testing can then be extrapolated to 'real time' conditions. Both real time and accelerated studies involve storing samples for periods of time under carefully controlled conditions and then performing specific analyses at different time points to monitor the development of oxidation and rancidity. This can be very time consuming and costly, but is sometimes necessary to fully evaluate the shelf stability of the product. This can be a barrier particularly with new product development or reformulation or changes in processing where a number of variants need evaluating and it is often impractical to carry out these long term evaluations at this stage of the products development. Under these circumstances, it is useful to have a rapid screening techniques that can give a quick indication of the relative performance of changes in formulation or processing and allows developers to narrow down the likely end formulation or process change before commencing a full shelf life study.

The following techniques are commonly used to assess oxidation shelf life stability including those that can speed up the process of shelf life assessment.

## **Rapid Techniques**

### **Oxidative Stability Index (OSI)**

As previously discussed in this paper, all oils and fats have a natural resistance to oxidation, which depends on certain factors including the degree of saturation of the fatty acids, level of antioxidants (natural or added) and the initial level of oxidised material that may be present (dependent on the quality of the original raw material). Oxidation proceeds slowly until resistance is overcome at which point it increases rapidly and the time taken before the rapid increase in oxidation occurs is called the 'induction period or induction time' which is used to make relative assessments on the oxidative stability.

The oxidative stability or resistance to oxidation of oils and fats can be measure using a Rancimat instrument. This is a technique that accelerates the oxidation process and monitors the level of oxidised volatile components released and can be used to determine the point at which the resistance has been overcome and the rapid increase in oxidation occurs.

The oil sample is aerated at elevated temperatures and the volatile oxidation products that are generated are captured in a vessel containing water and measured continuously via the increase in conductance of the water. The point of maximum change in the conductance and therefore the rate of oxidation are used to determine the induction time. This is the point at which the oils natural resistance to oxidation has been overwhelmed and there is a sudden increase in oxidation products and therefore an increase in conductivity. The longer the induction time the greater the resistance to oxidation.

The instrument has some limitations in that it can only really be used for oils and fats or certain food products that have a high fat content. However, it is particularly effective at assessing the relative quality of raw ingredient oils, comparing relative stability of oils/fats and blends of oils/fats and also comparing the relative efficiency of different added antioxidants and at different levels.

The stability of the oil assessed using the Rancimat is useful for the initial evaluation of raw materials but cannot be used on most finished products, however the oil might behave differently in the finished product compared to the oil/fat analysed and this is where another instrument, the Oxipres, can be particularly useful.

### **Oxidograph or Oxipres**

The Oxipres is an accelerated 'oxygen bomb' method that measure oxidative stability of oils, fats and finished product. Like the Rancimat, the Oxipres is an instrument that accelerates the oxidation process and works on a similar principle. The sample is placed in a pressurised cell which is filled with oxygen. The cell is held at elevated temperature (typically 80°C) and as the fat oxidises it consumes the oxygen and the pressure drops. There is a rapid decrease in pressure, and this point correlates to the rapid oxidation of the sample and increase in rancidity or 'induction time' when the resistance to oxidation has been overcome.

The Oxipres can be used to evaluate the effect of using different fats or antioxidants on oxidative stability in raw materials and finished products. It has the advantage of the analysis being conducted on the finished product in its final format - the sample can often be analysed whole without being ground or homogenised therefore allowing the impact of product format or structure to be incorporated.

Whilst the determination using the Oxipres cannot directly give the shelf life of the product it can give an indication relative to a control sample with a known shelf life - better or worse stability, thereby giving guidance on potential shelf life, which can be very helpful to rapidly screen changes in formulation or processing to determine the optimal possibilities.

## **Traditional Techniques for Measuring Oxidation**

Once product formulations or processing changes have been screened and narrowed down to a small number of candidates, these products can then be placed into carefully controlled storage conditions reflecting the conditions the product will be exposed to during its shelf life or harsher conditions to accelerate the oxidation process. At set time points samples can be taken and analysed to determine the impact on the sensory quality and level of oxidation. This can be carried out over time to build up a picture of the evolution of oxidised components and link this to acceptability of product

through sensory evaluation to guide the shelf life that can be assigned to a product.

Some of the methods for monitoring changes in oxidation are as follows:

### **Sensory Evaluation**

The use of sensory evaluation in assessing stability of products should not be underestimated. Sensory assessment can be completed by a consumer panel of untrained assessors who will assess based purely on 'like' or 'dislike'. This provides no information on sensory defects resulting from deterioration but reflects consumer acceptance of the products. On the other hand, trained assessors who are familiar with the product and possible perceptions resulting from sound or defective product can be beneficial. Using standardised vocabulary gives reproducibility and precision to sensory analysis. The combination of scientific analysis and sensory assessment is a valuable tool particularly when looking at the development of new products or reformulation of existing ones.

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### **Peroxide Value (PV) Determination**

The peroxide value is probably one of the most commonly used methods to measure the initial stages of oxidation of oils and fats. The peroxide value is often conducted using a titration based method to determine the level of iodine liberated from potassium iodide by the oxidised species in the sample but there are also colorimetric methods. Samples of oils and fats can be analysed directly using the peroxide value. However foods and finished products need to be extracted to recover the fat for the peroxide value determination. This extraction needs to be conducted very carefully to avoid further oxidation but to also ensure that the fat is sufficiently recovered from the finished product.

The peroxide value measures hydroperoxides that are produced in the early stages of the oxidation process. Care needs to be taken in interpreting the peroxide value results since the hydroperoxides readily degrade therefore samples with a low peroxide value can still have undergone significant oxidation. The peroxide value increases as the oil/fat oxidises but will decrease when the peroxides are degraded to secondary oxidation products such as aldehydes and ketones. Therefore it is important to combine peroxide value analysis that measures the initial products of oxidation with methods that measure the secondary products of oxidation such as the anisidine value. The combination of the peroxide value and anisidine value is referred to as the TOTOX value ( $2 \times$  the peroxide value + anisidine value) and is a useful measure of the initial and secondary oxidation products.

### **Anisidine Value**

This method is particularly good at detecting unsaturated aldehydes which are the ones that are most likely to generate unacceptable flavours. As described above this method is useful in combination with the peroxide value to give an overall picture of the oxidation of oils or fats.

p-Anisidine reacts with secondary oxidation products such as aldehydes and ketones in fats and oils to form products that absorb at 350 nm wavelength.

The method is generally applied to pure oils and fats and can suffer from interferences if an oil or fat is extracted from a food product due to the co-extraction of components that react with the anisidine.

### **Thiobarbituric Acid Analysis (TBA)**

The TBA analysis is another method that is commonly used and is a colorimetric method that is used to measure the increase in red pigment formed in the reaction of 2-thiobarbituric acid (TBA) and oxidised lipids. Malonaldehyde (and other aldehydes) produced through oxidation react with the TBA reagent and are measured spectrophotometrically. The TBA number is calculated as mg of malonaldehyde per kg of sample. The method is not specific and potentially results in interferences, however it is useful as the whole sample can often be analysed.

### **Gas Chromatography Mass Spectrometry (GC-MS) for Aldehydes**

This method directly measures the individual components that are the cause of flavours and can be linked to sensory evaluation. There are several ways of introducing the sample into the instrument and a technique more commonly used involves solid phase micro extraction (SPME) GC-MS. These methods can be very sensitive but do involve the use of expensive instrumentation and require experienced analysts to operate and interpret the data.

### **Conjugated Dienes**

The different stages of oxidation can involve the formation of conjugated dienes and trienes and these can be measured spectrophotometrically at 232 nm and 270 nm. The method is simple and involves dissolving a small amount of oil or fat in iso-octane and measuring the absorbance in a UV spectrophotometer.

As described above, there are a number of tools available that can be used to assess the oxidation state of oils, fats and finished products at a particular point in time or to accelerate and monitor the oxidation process. The use of these methods and instruments has been demonstrated as beneficial in product development and shelf life as described below.

## Case Study Examples

### Shelf Life Extension

A common desire of food manufacturers is to extend the shelf life of products. One product development challenge involved the use of antioxidants to improve the oxidative stability but also maintain a 'natural' ingredients label. In this case, the product developers were interested in evaluating the effects of natural antioxidants based on plant extracts such as green tea and rosemary extract. They then wanted to compare the efficiency of these relative to the more common and effective synthetic antioxidants BHT and BHA.

Since the number of possible combinations of antioxidants and concentrations was quite large, a screening exercise was conducted by adding different levels of the antioxidants to the base oil used in the recipe and evaluating these compared to BHT using the Rancimat instrument. After several trials of differing antioxidants, concentrations and combinations, the more promising candidate oil/antioxidant blend was incorporated into finished product on a laboratory/pilot scale.

This prototype finished product formulation was evaluated directly using the Oxipres as a rapid accelerated evaluation of the oxidative stability and compared to the existing formulation. After a slight adjustment of the concentrations used, a more long term study was initiated by storing the sample in controlled conditions that mimicked real storage conditions and conducting sensory analysis, peroxide value and TBA determination at different time points during the shelf life. The development of any off flavours/notes or oxidised components was monitored over time. The products were also stored at elevated temperatures and evaluated at more frequent time points to gain a quicker insight into the products performance and provide guidance on whether the product was likely to meet the goal of increased shelf life.

This approach enabled the developer to screen many formulation options in a much shorter time frame at relatively lower cost compared to assessing the product in the real life conditions. It also gave some confidence on performance before starting a more time-consuming stability trial.

### Fat Reformulation – Saturated Fat Reduction

There has been much publicity in the media about the negative impact of saturated fat on health, although this has been questioned more recently. Many manufacturers however are still targeting a reduction in the amount of saturated fat in their products to fulfil health and wellness commitments. One manner of achieving this is to substitute a portion of the saturated fat, in this case from palm

oil, in the recipe with rapeseed oil. There were, however, concerns about the stability of the final fat blend since rapeseed contains a significant amount of unsaturated and polyunsaturated fatty acids that are particularly prone to oxidation.

A similar approach to that previously described was used to assess the stability of different blends of the oils and fats using the Rancimat instrument which resulted in the incorporation of antioxidants to meet the required shelf life. The resulting formulation in the finished product was compared to the original formulation with a known shelf life using the Oxipres instrument to give confidence that the new formulation would perform at least as well as the original formulation.

### Monitor Susceptible Ingredients

Many food manufacturers have sought to incorporate food ingredients into their products with the aim to use a positive nutritional or health claim. The omega 3 fatty acids have been used in the development of many new products which has been driven by the positive EFSA approved health claims associated with this ingredient. The use of omega 3 fatty acids does however generate a number of challenges, not least the oxidative stability of the ingredient.

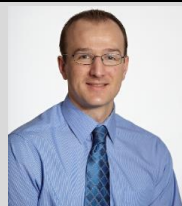
To increase the stability of finished products, developers have incorporated these ingredients into their products as emulsions with added antioxidants and also as encapsulated oils. The encapsulation reduces the exposure of the ingredient to air and reduces the level of oxidation. Development of products incorporating these ingredients has involved the assessment of the quality and stability of the initial raw material ingredients which could be from different sources such as fish oils, algal oils or from vegetable oils. The use and effectiveness of antioxidants has also been monitored in addition to the impact of incorporating the ingredient as an emulsion or as encapsulated oil. These developments have also been assessed using the rapid accelerated techniques described above to evaluate the oxidative stability before the finished products have been placed into real time shelf life studies to monitor the level of oxidation using the methods already described.

### Summary

Oxidation of oils and fats has a critical influence on the shelf life of ingredients and finished products. However, there are several strategies available to improve the oxidative stability and therefore shelf life of products.

To aid product development and reformulation, there are many tools that can be used to monitor oxidation and oxidative stability in oils, fats and finished

products. These can be extremely useful to ensure that a product maintains the level of quality expected by the consumer over the shelf life of the product. There are many drivers for reformulation of products or changes to the production process and some of the tools described in this paper can provide rapid guidance to the likely impact on the products stability. It is often the case that one method is not sufficient to assess the stability alone, but it is the use of some of these complementary techniques at different stages of product development that can speed up the development process and offer a quicker route to market with the confidence that the new product will meet the consumer expectation throughout its shelf life.



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