IFFO Fishmeal stability trial

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Summary

IFFO, with the support of one of its producer members, TASA, conducted a fishmeal stability trial on reactive anchovy fishmeal to determine the stability of fishmeal dosed with ethoxyquin at levels lower than normal as well as with the available alternative antioxidants. Fishmeal was stored at ambient temperatures in a warm climate (Peru) for a period of 18 months in 50 kg as well as 1 ton bags and were dosed with the lower ethoxyquin levels of 300 ppm and roughly 50 ppm and the alternative antioxidant treatments were 440 and 860 ppm BHT and the natural tocopherol and rosemary extract blend (390 and 630 ppm tocopherol). The results showed that lower dosage levels of ethoxyquin as well as the alternative antioxidants provide effective protection of fishmeal during long-term storage at ambient temperatures for both 50kg and 1 ton bags. The protection of the valuable omega-3 fatty acids, which in many instances distinguishes fishmeal from other feed ingredients, is desirable and 300ppm ethoxyquin, as well as the low ethoxyquin dosage level of 50ppm, have proven to be extremely effective in protecting the valuable omega-3 fatty acids, in fact more so than any of the alternative antioxidants evaluated in the trial.

In addition, there were ample residual antioxidants remaining (> 35% in 50kg bags and >45% in 1ton bags) after 18 months storage to provide further protection for at least another 6 months.

The results were successfully used as support for amendments to the Model Regulations for the shipping of Dangerous Goods of the United Nations Transport for Dangerous Good committee where the reduced residual ethoxyquin level (50ppm instead of current 100ppm) as well as the inclusion of the alternative antioxidants BHT with a 100ppm residual level and natural tocopherols with a 250 ppm residual level was accepted into the Model Regulations.

Introduction

Fishmeal containing high levels of polyunsaturated fatty acids and fat content needs to be stabilised by the addition of an antioxidant to prevent oxidation that could result in spontaneous combustion. Heat is generated when there is rapid and significant oxidation of fishmeal due to the exothermic reaction of oxygen with the highly polyunsaturated fatty acids (in particular eicosapentaenoic, EPA and docosahexaenoic acids, DHA) resulting in the spontaneous combustion of fishmeal.

It is a legal requirement of the International Maritime Organisation (IMO) to add an antioxidant to fishmeal - prior to shipping – to ensure safe transportation and storage of the raw material and prevent the spontaneous combustion of fishmeal during shipping and storage. Fishmeal has been stabilised by the addition of the antioxidant, ethoxyquin (EQ) for many years and it is estimated that roughly 66% of globally-traded fishmeal contains ethoxyquin. The current dosage level, along with the required residual levels at the time of consignment, of ethoxyquin as it is listed in the IMDG Code were determined more than 40 years ago, and are likely to be at levels well in excess of those that will achieve stabilisation, having been based on the information which was available at that time. Unnecessarily high levels of ethoxyquin are undesirable and may lead to high residue levels in the animal which has been fed feed which incorporates the treated fishmeal as a feed ingredient. At the time of writing the level of EQ and other synthetic antioxidants are controlled through the setting of a maximum level in feed (150 mg/kg) in the EU, but there remains an opportunity to optimise the use of EQ and reduce levels in the supply chain.

Increased negative publicity led to an increasingly negative general perception of ethoxyquin in the EU and along with difficulties with high levels of ethoxyquin found in shrimp originating from Asian countries exporting to Japan, attention has been drawn on to the use of ethoxyquin. In addition, the potential carry-over of fat soluble ethoxyquin into omega-3 oils produced from by-products of farmed fish may be a cause for concern. There are strong drivers to reduce the levels of ethoxyquin present in animal feeds, and are also drivers for optimising the use of ethoxyquin in the stabilisation of fishmeal.

Ethoxyquin has been evaluated for re-authorisation as a feed additive according to the requirements of European Parliament and Council Regulation (EC) No 1831/2003, that sets out new rules for the authorisation, supervision and labelling of feed additives. All feed additives authorised on the EU market had to apply for re-authorisation if they wanted to stay in the market. Application dossiers were requested to be submitted to the EU authorities by November 2010, and feed additives are being processed progressively, including EQ. EFSA reported their Opinion on EQ in November 2015 (EFSA, 2015) which was inconclusive with regards to the safety of ethoxyquin. The European Commission has published the Implementing Regulation in June 2017 that partially suspends the authorisation of ethoxyquin as a feed additive for all animal species and categories with some derogations for marine ingredients (e.g. fishmeal) and vitamin blends in which some transitional (phasing out) provisions are provided. The reauthorisation process has been extended to allow for the provision of additional safety studies required to make the full assessment.

The increasing negative perception of ethoxyquin along with the possibility that EQ may not be re-authorised after the safety evaluation conducted by EFSA, led IFFO to plan and conduct fishmeal stability trials to ascertain whether lower levels of ethoxyquin will effectively protect fishmeal and at the same time evaluate the efficacies of a synthetic and a natural antioxidant alternative. BHT is currently the only possible alternative antioxidant written into the IMDG code and is a synthetic antioxidant that is also undergoing a similar re-authorisation process that ethoxyquin is going through. BHT was included in the study as the alternative synthetic antioxidant option to ethoxyquin which will be less costly than natural alternatives. The antioxidant levels used was similar to the current ethoxyquin dosage levels and provided an indication of the comparative efficacies. The natural antioxidant often used in the pet food market is a tocopherol/ rosemary extract blend. Including the natural, although more pricey option, will once again provide producers with an indication of the comparative efficacies of the different existing antioxidants. The scientific data produced from the trial were to be presented to the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods and IMO that could potentially result in lower residual ethoxyquin levels at time of consignment and the inclusion of tocopherols as an alternative antioxidant option into the IMO shipping codes.

Trial methodology

The fishmeal stability trial was planned with support from producers in the fishmeal industry (TASA, Peru) and antioxidant distributors (Kemin and Rheintek). The proposed plan of the fishmeal stability trial had been presented to the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods to ensure that the trial will provide the necessary information to permit changes to the shipping rules of fishmeal. The sub-committee provided comment and had additional requirements such as storing larger volumes of fishmeal (1 ton bags), storing the fishmeal at higher temperatures to mimic conditions during shipping, regular ambient temperature measurement along with measurement of fishmeal temperature and performing the self-heating test. The requests were included into the trial where possible although it was not possible to store such large volumes of fishmeal at elevated temperatures up to 50°C and fishmeal was stored in the warehouse under ambient conditions in the warm Peruvian climate. Peruvian anchovy fishmeal was chosen as a representative of a meal containing higher levels of polyunsaturated fatty acids and therefore possessing a more reactive capability.

Objectives

- To determine if a lower dosage level of EQ will provide long term protection of fishmeal
- To determine the efficacies of available alternative antioxidants and the recommended dosage levels

Trial detail

The same batch of highly polyunsaturated reactive anchovy fishmeal anchovy meal were treated with the antioxidants directly after production, as is standard practice in the industry. Fishmeal was taken from the main production line before the addition of antioxidants. The antioxidants were added in a pilot scale line after which it was bagged and stored under similar conditions that fishmeal is normally handled in the factory.

The following fishmeal treatments were conducted:

- Fishmeal dosed with 600 mg/kg EQ (current general dosage level)
- Fishmeal dosed with 300 mg/kg EQ (below minimum IMO level of 400 mg/kg)
- Fishmeal dosed with 2,000 ppm BHT **solution** (alternative synthetic antioxidant)

- Fishmeal dosed with 4,000 ppm BHT **solution** (alternative synthetic antioxidant)
- Fishmeal dosed with 2,000 ppm tocopherols/rosemary extract blend (alternative natural antioxidant)
- Fishmeal dosed with 4,000 ppm tocopherols/rosemary extract blend (alternative natural antioxidant)

Each treatment was stored in 50kg as well as 1 ton bags and were stacked together. These volumes represent standard production packages. The storage facility was safe and secure. The ambient temperature was monitored throughout the trial along with the temperature of the fishmeal for each treatment. Representative samples were taken during storage by inserting a sampling stick into the sacks in various points.

The sampling intervals were: Day 0, Week 2, Month 2, Month 6, and Month 12. The following analyses were performed throughout the trial:

- Antioxidant content (AO): Ethoxyquin, BHT, tocopherol
- Peroxide value (PV): measurement of primary oxidation
- Anisidine value (AV): measurement of secondary oxidation
- Free fatty acids (FFA): measurement of hydrolytic activity – enzymes
- Omega-3 content (PUFA): indication of oxidative deterioration of fatty acids
- Self-heating test: determination whether a substance have the ability of to undergo oxidative self-heating and thereby spontaneously ignites

The decrease in the total omega-3 fatty acid content is a very useful measure of the efficacy of antioxidants in fishmeal (De Koning, 1998). Due to their highly unsaturated nature the omega-3 fatty acids are very susceptible to oxidation and the omega-3 content will decrease if oxidised. The protective effect of antioxidants that prevents the oxidation of the polyunsaturated fatty acids can clearly be seen after longer storage periods.

A Schaal Oven Test conducted at 60°C that allows for the accelerated evaluation of antioxidants and the stability of fishmeal was conducted on a different batch of fishmeal using the same treatments. Due to the relatively low temperature the deterioration of fishmeal can be correlated with ambient temperatures. Although the results for this test at this stage was inconclusive it may be an option for the future evaluation of antioxidant in fishmeal.

The treatment and analyses plan for the long-term fishmeal stability trial can be seen in Table 1 along with the theoretical contents of the active components (antioxidants) in the treatments calculated from the percentage in the dosing solution according to the specifications.

The specifications of the antioxidants used in the trial are as follows[1]:

- Ethoxyquin (EQ): Minimum 95% solution;
- Naturox Premium liquid: 23.2% tocopherols and <1% rosemary extract (containing ≈ 5% carnosic acid);
- Rendox T: 20% BHT solution.

[1] It is interesting to note that the IMO regulations do not specify the active content of the antioxidant used, but refers merely to the additive in the generic sense. The antioxidants used in the trial were representative of those available on the market and in use in the industry.

Table 1: Antioxidant treatments, theoretical content of the active component and analyses plan

Treatments	Theoretical antioxidant content (active component) (ppm)	Sampling intervals				
		Day		Month		
		0	14	2	6	12
EQ: 300 ppm	285	AO, PV, AV, FFA, PUFA	AO, PV, AV, FFA.	AO, PV, AV, FFA	AO, PV, AV, FFA. Self-heating test (50 kg bags only)	AO, PV, AV, FFA, PUFA Self-heating test (all sizes)
EQ: 600 ppm	570					
BHT solution: 2,000 ppm	400					
BHT solution: 4,000 ppm	800					
Tocopherol/ rosemary extract blend: 2,000 ppm	460 ppm tocopherols + < 20 ppm rosemary extract (containing 1 ppm carnosic acid)					
Tocopherol/ rosemary extract blend: 4,000 ppm	920 ppm tocopherols + < 40 ppm rosemary extract (containing 2 ppm carnosic acid)					

Where AO = antioxidant; PV = Peroxide value; AV = Anisidine value; FFA = Free Fatty acids

The 1 ton bags were only analysed intermittently due to cost restraints.

Methods of analysis

The analyses were performed using the following methods:

- Ethoxyquin: SGS-INO-ME-06: 2016, Rev.07 (Validated) Fishmeal: Determination of Ethoxyquin by High Performance Liquid Chromatography
- Tocopherol: SGS-INO-ME-44:2014; REV00. Fishmeal: Total tocopherols by High Performance Liquid Chromatography.
- BHT: AOAC 983.15:2016; 20th Ed., Phenolic Antioxidants in Oils, Fats and Butter Oil.
- Fatty acids: AOCS Ce 1b-89: 2009; 6th Edition. Fatty Acid Composition of Marine Oils by GLC.
- Peroxide value: SGS-PO-ME-81: 2016, Rev.05 (Validated) Fishmeal: Determination of index Peroxide
- Anisidine value: AOCS Cd 18-90: 2009; 6th Ed.
- Free fatty acids: SGS-PO-ME-43: 2016, Rev.06 (Validated) Fishmeal. Determination of Free Fatty Acids. (Cold Extraction).
- Protein (Dumas): AOAC 990.03 : 2012 ; 19th Ed. Protein (Crude) in animal feed
- Fat: SGS-INO-ME-14 :2013; Rev.02; Fishmeal: Determination of Moisture, Fat and Ash by Near Infrared Spectroscopy.
- Moisture: SGS-INO-ME-14: 2013; Rev.02; Rev. 01. Fishmeal: Determination of Moisture, Fat and Ash by Near Infrared Spectroscopy.
- Ash: AOAC 942.05: 2012; 19th Ed. Ash of animal feed.
- Salt: SGS-INO-ME-39. Rev00:2012. Sodium Chloride in Fishmeal - Potentiometric Method
- TVN: NTP 201.032:1982. (Reviewed 2010). Meat and Meat Products. Determination of Ammoniacal Nitrogen Content
- Histamine: SGS-INO-ME-07 Rev. 07: 2010; Hydrobiological Products: Determination of Histamine by High-Resolution Liquid Chromatography.
- Oxygen Bomb test:

In addition, the oxygen bomb test was performed once after around 6 months' storage. The Oxygen Bomb Test is used to predict stability and evaluate antioxidant systems in fats and finished products. The test is performed in a closed system at 98°C which determines the drop in the oxygen pressure. As the polyunsaturated fatty acids in the fishmeal oxidises it uses oxygen and the oxygen pressure decreases. The period that the fishmeal resists oxidation is the induction period and the shorter the induction period the less stable the fishmeal is. Therefore, fishmeal that oxidised quickly will have a shorter induction period as the oxygen pressure drops more rapidly.

Results and Discussion

The various treatments provided a range of data from which an overall analysis has been undertaken. All treatments showed a decline in antioxidant content over time. It was highlighted that a problem had occurred during the dosage of the 600ppm ethoxyquin treatment (50kg and 1 ton) and the 50kg fishmeal bags only contained on average roughly 30ppm ethoxyquin whereas the 1 ton fishmeal bags contained on average around 50 ppm ethoxyquin. Those treatments were retained in the trial in order to provide an assessment of the performance of the antioxidant at very low dose.

Proximate analyses

The composition for each treatment of fishmeal was determined at the start of the trial and can be seen in Table 2.

Table 2: Proximate analysis for the different fishmeal treatments at start of the trial

Antioxidant treatments	Storage bag size	Proximate analyses						
		Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Salt (%)	Histamine (mg/kg)	TVBN (mgN/100g)
Ethoxyquin: 320 ppm	50 kg	70.1	7.7	7.7	15.4	1.9	73	61
Ethoxyquin: 300 ppm	1 ton	69.2	7.7	7.7	15.6	1.7	72	61
Ethoxyquin: 30 ppm	50kg	Not available	Not available	Not available	Not available	Not available	Not available	Not available
Ethoxyquin: 50 ppm	1 ton	Not available	Not available	Not available	Not available	Not available	Not available	Not available
BHT: 440 ppm	50 kg	70.1	6.6	7.7	15.2	1.8	76	65
	1 ton	70.1	7.0	7.7	15.5	1.8	63	61
BHT: 860 ppm	50 kg	69.4	6.8	7.8	15.3	2.0	53	64
	1 ton	69.4	7.0	7.9	15.3	1.9	55	61
Natural blend: 390 ppm	50 kg	69.2	7.6	7.7	15.5	1.7	35	68
	1 ton	69.2	7.6	7.4	15.3	1.8	38	62
Natural blend: 630 ppm	50 kg	70.1	7.4	7.5	15.3	2.0	46	61
	1 ton	69.6	7.4	7.6	15.4	1.8	39	64

The fishmeal used in the trial was of good quality with a high protein content (> 69%) with a fat content around 7.7%, ash 15.4% and salt 1.8%. The histamine and Total Volatile Base Nitrogen (TVBN) contents were low indicating fresh raw material and good quality fishmeal. However, it is important to note that most of the biogenic amines (which includes histamine) and TVBNs end up with the fish solubles (stickwater) during processing and the amount of these in the fishmeal are dependent on the

amount of stickwater that was returned to the presscake. The composition of the fishmeal could also be slightly different dependent on whether the stickwater has been return to the presscake.

Antioxidant content

The various antioxidant contents were measured throughout the trial. The residual antioxidant (AO) contents can be seen in table 3.

Table 3: Initial antioxidant contents and residual antioxidant contents determined during storage

Antioxidant treatment	Storage bag size	DAY			MONTH				
		Mean 0	0	0	0.5	2	6	12	18
Ethoxyquin: 320 ppm	50 kg	318	328	307	322	282	259	151	117
Ethoxyquin: 300 ppm	1 ton	297	284	310		240		213	171
Ethoxyquin: 30 ppm	50kg	29	27	30	31	32	<15	13	ND
Ethoxyquin: 50 ppm	1 ton	48	51	44		55		36	28
BHT: 440 ppm	50 kg	438	434	442	476	346	368	290	279
	1 ton	438	441	435		377		342	412
BHT: 860 ppm	50 kg	858	837	879	865	714	751	659	518
	1 ton	867	875	858		776		775	673
Natural blend: 390 ppm	50 kg	385	374	396	344	338	243	209	151
	1 ton	400	389	411		368		280	189
Natural blend: 630 ppm	50 kg	628	626	630	566	562	488	277	248
	1 ton	752	749	755		791		599	421

The percentage residual antioxidant content can be seen in Table 4.

Table 4: Percentage residual antioxidant content after 6, 12 and 18 month's storage.

Antioxidant treatment	Storage bag size	Residual antioxidant content (%)		
		6 months	12 months	18 months
Ethoxyquin: 320 ppm	50 kg	81.4	47.6	36.9
Ethoxyquin: 300 ppm	1 ton		71.7	57.6
Ethoxyquin: 30 ppm	50kg	< 53.6	< 45.6	ND*
Ethoxyquin: 50 ppm	1 ton		75.8	58.9
BHT: 440 ppm	50 kg	84.0	66.2	63.7
	1 ton		78.1	94.1
BHT: 860 ppm	50 kg	87.5	76.8	60.4
	1 ton		89.4	77.7
Natural blend: 390 ppm	50 kg	63.1	54.3	39.2
	1 ton		70.1	47.3
Natural blend: 630 ppm	50 kg	77.7	44.2	39.5
	1 ton		79.6	56.0

* Not detected

Figure 1 shows the decrease in antioxidant content during storage.

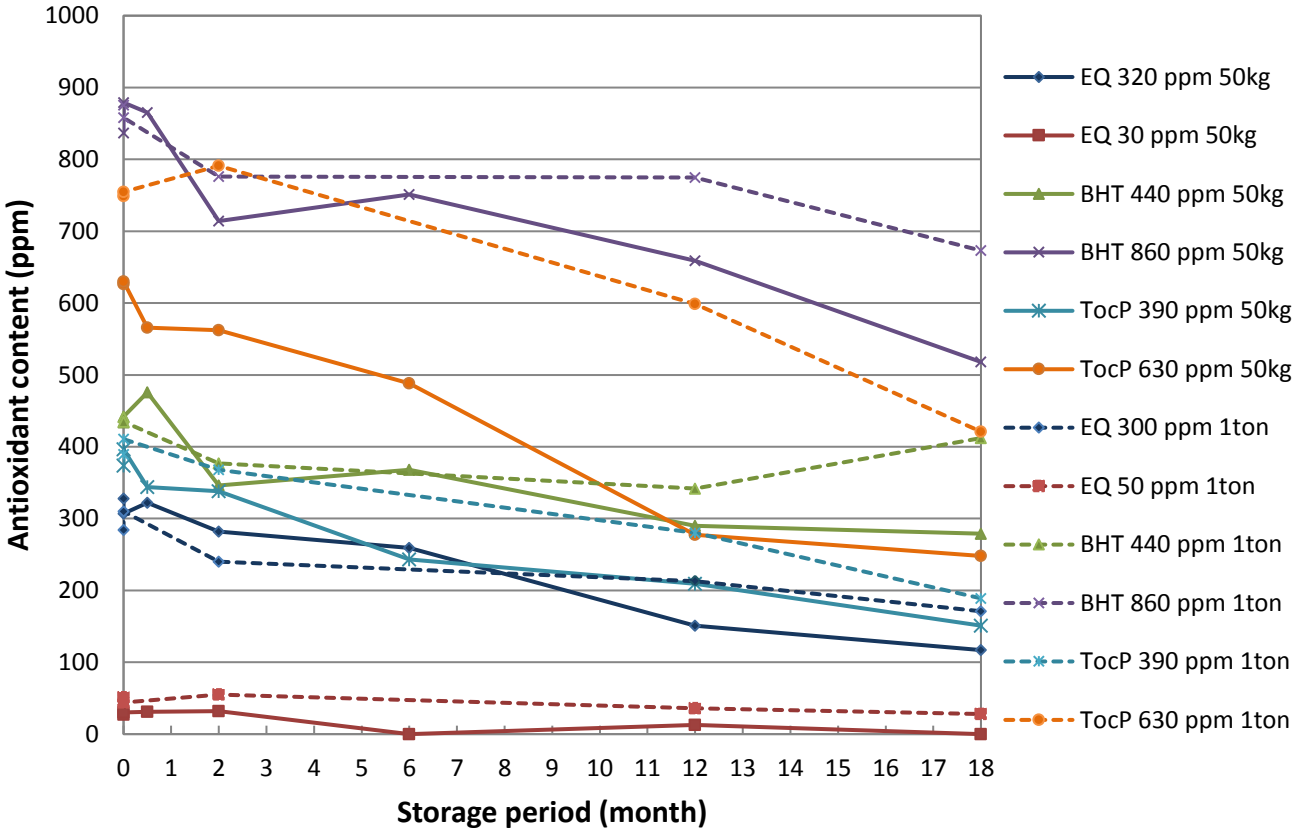

Figure 1: Decrease in antioxidant levels in fishmeal treated with different antioxidants stored for 18 months.

The results show that the antioxidant levels after 12 months (and 18 months) of storage have decreased to levels that are still sufficient to provide continued protection to the fishmeal. It appears that the antioxidant levels in the 1 ton bags decreased at a slower rate indicating less consumption of the antioxidant and therefore potentially a slower rate of oxidation. The antioxidant levels after 12 months storage in the 50 kg bags decreased in order from fastest to slowest by < 54.4% (ethoxyquin 30 ppm), 55.8% (Natural blend 4,000 ppm), 52.4% (ethoxyquin 300 ppm), 45.7% (Natural blend 2,000 ppm), 33.8% (BHT 2,000 ppm) and the slowest decrease of 23.2% (BHT 4,000ppm). Similarly, the antioxidants in the 1 ton bags decreased by 29.9% (Natural blend 2,000 ppm), 28.3% (ethoxyquin 300 ppm), 24.2% (50 ppm ethoxyquin), 21.9% (BHT 2,000 ppm), 20.4% (Natural blend 4,000ppm) and 10.6% (BHT 4,000ppm). The highest decrease in antioxidant of roughly 55% still leaves sufficient antioxidant remaining to protect fishmeal for another period of up to 6 months or more after 12 month's storage. Even after 18 months storage sufficient antioxidant content (> 35% for the lowest remaining percentage) remained to provide further protection.

The results of the residual antioxidant contents also indicated that for each treatment the antioxidant levels in the 1 ton bags were consistently higher than in the 50kg bags where all of the 1 ton bags showed higher percentage residual antioxidants for all the treatments. The higher residual levels could be due a slower rate of oxidation in the big bags but a slower deterioration rate in the big bags has not been confirmed.

Oxygen Bomb test

The oxygen bomb test was performed on the samples after roughly 6 months of storage. The results can be seen in Table 5 and Figure 2. It is a useful comparison test of antioxidant efficacies which clearly shows that the 300ppm ethoxyquin performed better than any of the alternative antioxidants with an

induction period of 9.5 hours. The 30ppm ethoxyquin treatment showed the lowest induction period of 1.2 hours but the 50ppm ethoxyquin treatment had an induction period of 2.7 hours that is similar to BHT. The induction periods of the BHT treatments ranged between 2.4 -2.9 hours which is considerably less than the 300ppm ethoxyquin treatments. The induction periods of the natural tocopherol blend treatments ranged between 2.5 -3.8 hours which is surprisingly slightly higher than the BHT treatments but still considerably less than the 300ppm ethoxyquin treatments. Although an useful test as indicator it cannot be correlated to real conditions because the oxidation reactions at the elevated temperature of 98°C is different than those at ambient temperatures.

Table 5: Oxygen bomb test results (hrs) after roughly 6 months of storage

Oxygen bomb induction period measured in hours		
Treatments	**Storage bag size**	**Induction period (hrs)**
Ethoxyquin: 320 ppm	50 kg	9.5
Ethoxyquin: 300 ppm	1 ton	9.5
Ethoxyquin: 30 ppm	50kg	1.2
Ethoxyquin: 50 ppm	1 ton	2.7
BHT: 440 ppm	50 kg	2.4
	1 ton	2.8
BHT: 860 ppm	50 kg	2.6
	1 ton	2.9
Natural blend: 390 ppm	50 kg	2.5
	1 ton	3.4
Natural blend: 630 ppm	50 kg	3.8
	1 ton	3.5

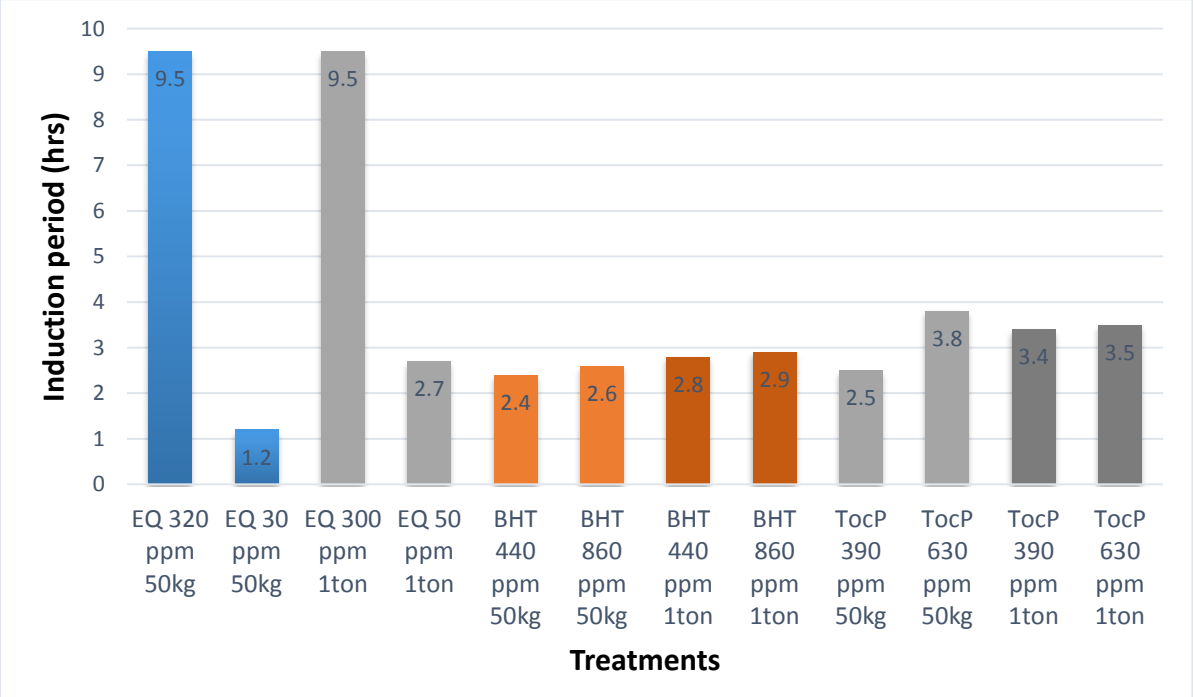

Figure 2: Comparison of oxygen bomb test results of the different antioxidant treatments after roughly 6 months of storage.

Free fatty acids (FFA)

Free fatty acids determine hydrolytic rancidity due to the hydrolysis of fatty acids from the glycerol to form free fatty acids mainly due to enzymatic activity before processing.

The FFA results of the different antioxidant treatment for a period of 18 months storage can be seen in Table 6 and Figure 3. The initial FFA values are high due to enzyme activity before processing and hydrolysis that occurs during processing. The FFA value for all the treatments increased gradually during storage. Of all the treatments, the 320 and 300 ppm ethoxyquin treatments showed the lowest FFA increase after 18 months (5.43 and 26.2%, respectively) with the highest FFA increases seen in the tocopherol treatments of ranging between 42.0 % and 72.5%. The FFA values for the BHT treatments increased by between 7.8% and 62.4%. There was significant variation between the percentage increases in FFA values for the different antioxidant treatments which could lead to unreliable interpretation of the results when looking at differences between each antioxidants' dosage level and storage volume.

Table 6: The FFA values of the different antioxidant treatments during 18 months storage period.

Antioxidant treatment	**Storage bag size**	**Free fatty acids (g/100g)**					
		DAY	**MONTH**				
		0	**0.5**	**2**	**6**	**12**	**18**
Ethoxyquin: 320 ppm	50 kg	4.42	4.02	3.90	3.98	5.23	4.66
Ethoxyquin: 300 ppm	1 ton	3.55		3.5		3.76	4.48
Ethoxyquin: 30 ppm	50kg	3.57	4.11	3.70	3.94	5.21	4.72
Ethoxyquin: 50 ppm	1 ton	3.18		2.9		3.96	4.28
BHT: 440 ppm	50 kg	3.54	4.7	4.30	4.22	4.49	4.17
	1 ton	3.59		3.30		4.19	3.87
BHT: 860 ppm	50 kg	3.23	4.03	4.00	3.83	4.03	4.37
	1 ton	3.03		3.60		3.74	4.92
Natural blend: 390 ppm	50 kg	3.41	4.08	3.50	4.01	4.64	5.27
	1 ton	3.28		3.20		4.73	4.66
Natural blend: 630 ppm	50 kg	3.27	4.05	4.10	4.26	4.54	4.80
	1 ton	3.06		2.90		4.23	5.28

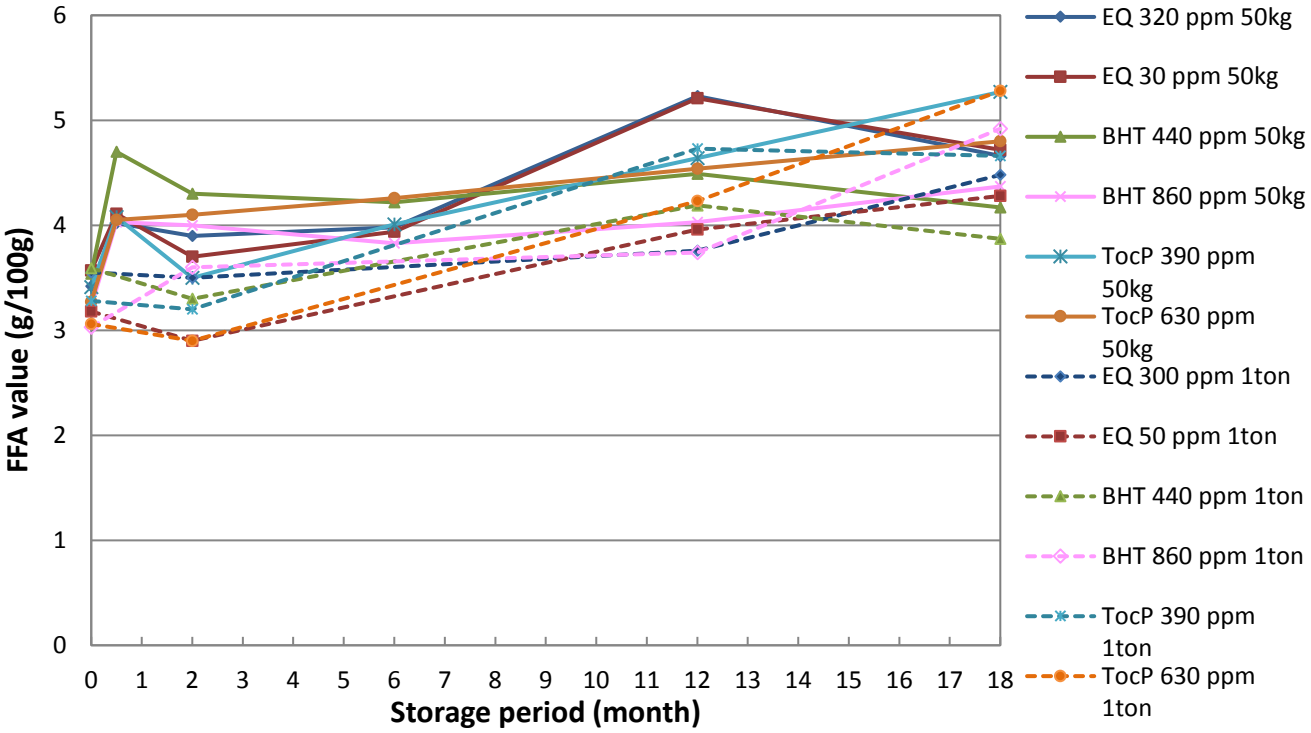

Figure 3: Change in FFA in fishmeal for the different antioxidant treatments during 18 months storage.

Peroxide value (PV)

The peroxide value determines the initial stage of oxidation (primary oxidation) and measures hydroperoxides that are produced in the early stages of the oxidation reactions. The hydroperoxides are subsequently broken down or degraded to secondary oxidation products such as aldehydes and ketones and therefore the PV will decrease over time indicating a progression in oxidation. The PVs on their own as indication of oxidation or quality of fats and oils can therefore be misleading and other oxidation indicators should also be taken into account when considering the oxidative status of a product.

The PV results can be seen in Table 7 and Figure 4. The initial PV was high as fishmeal oxidised rapidly during and after production where temperatures up to 90°C are reached. The PVs decreased as the hydroperoxides formed during production was broken down to secondary oxidation products and the antioxidants started to react with the free radicals thereby slowing down the oxidation. After 6 months' storage, the PV's slowly increases again with continued oxidation and here a difference can be seen between 300ppm ethoxyquin treatment and the tocopherols where ethoxyquin provides more protection against continued oxidation than tocopherols. The highest PV reached after 12 months storage period was 19 meq/kg which was for the 630 ppm (1 ton) natural tocopherol treatment where the lowest PV of 2.6 and2.5 meq/kg after 12 months storage was for the 300 ppm ethoxyquin (1 ton kg) and 50ppm ethoxyquin (1 ton) treatments, respectively. The PVs generally decreased again after 12 months's storage, indicating a breakdown of the primary oxidation products to further oxidation products. Although the tocopherol blends seem to have slightly higher PVs than the ethoxyquin (and BHT) treatments, the PVs were relatively low for all treatments during the 18 month's storage period.

Table 7: The PV's of the different antioxidant treatments during 18 months storage period.

Antioxidant treatment	Storage bag size	Peroxide value (meq/kg)					
		DAY	MONTH				
		0	0.5	2	6	12	18
Ethoxyquin: 320 ppm	50 kg	28.9	9.9	8.0	5.1	4.5	1.8
Ethoxyquin: 300 ppm	1 ton	27		6.0		2.6	2.3
Ethoxyquin: 30 ppm	50kg	26.5	14.3	7.0	10.2	18.0	6.1
Ethoxyquin: 50 ppm	1 ton	44.3		7.0		2.5	3.5
BHT: 440 ppm	50 kg	36.1	24.2	7.0	6.8	7.9	5.3
	1 ton	40.1		10.0		3.0	5.3
BHT: 860 ppm	50 kg	34	17.9	13.0	6.3	8.0	4.6
	1 ton	31.5		9.0		4.5	4.9
Natural blend: 390 ppm	50 kg	28.5	30.2	9.0	11.8	8.2	6.6
	1 ton	42.6		10.0		17.2	11.0
Natural blend: 630 ppm	50 kg	43.1	37.7	11.0	17.4	11.2	9.8
	1 ton	41.9		12.0		19.0	13.3

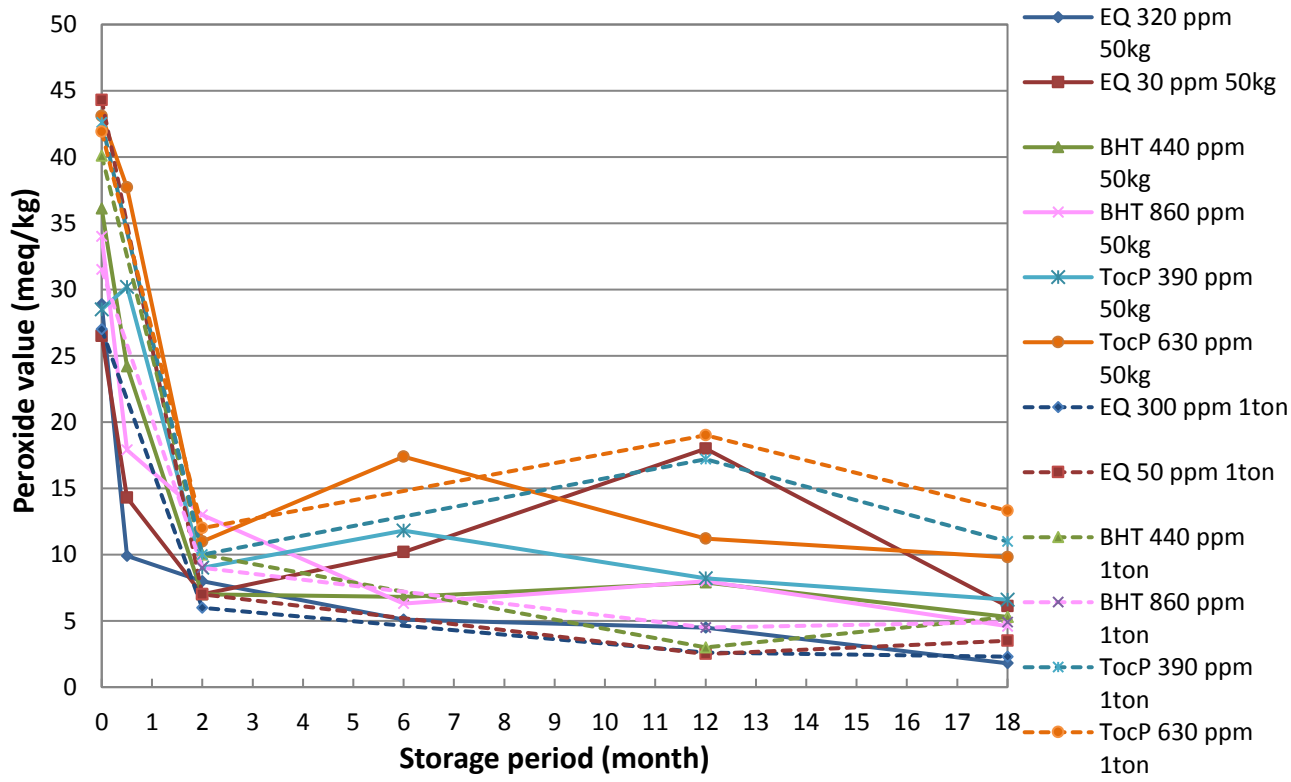

Figure 4: Change in PV in fishmeal for the different antioxidant treatments during 18 months storage.

Anisidine value (AV)

The AV determines secondary oxidation products such as aldehydes and ketones. The AV results can be seen in Table 8 and Figure 5. The initial AVs were high because of the breakdown of the initial high PVs to secondary oxidation products as measured by AV. The high AVs are also possibly due to discoloration of the extracted oil that occurs during the heat treatment, (also from the concentrated

antioxidant treatments) or co-extraction of interfering components that reacts with the *p*-anisidine, which will interfere with the absorbance measurement. The highest AV reached during the 18 months storage period was 177 for the 390 ppm (1 ton) natural tocopherol treatment whereas the lowest AVs after 18 months storage of 37, 42 and 47 were for the 320 ppm ethoxyquin (50 kg), the 30 ppm ethoxyquin (50 kg) and the 440 ppm (50kg) BHT treatments, respectively. The AV's were relatively stable up to 12 months of storage but after the 18 months storage most of the values showed a significant increase indicating accelerated oxidation. The decrease of the AVs for the 320 ppm ethoxyquin (50 kg), the 30 ppm ethoxyquin (50 kg) and the 440 ppm (50kg) BHT treatments at 18 months is hard to explain. The tocopherol blends and the BHT treatments in general seem to have slightly higher AVs than the ethoxyquin treatments, and it also appears that the 50 kg bags had lower AV values than the 1 ton bags.

Table 8: The AV's of the different antioxidant treatments during 18 months storage period.

Antioxidant treatment	**Storage bag size**	**Anisidine Value**					
		DAY	**MONTH**				
		0	**0.5**	**2**	**6**	**12**	**18**
Ethoxyquin: 320 ppm	50 kg	129	85	41	47	63	37
Ethoxyquin: 300 ppm	1 ton	157		40		58	99
Ethoxyquin: 30 ppm	50kg	121	106	75	58	75	42
Ethoxyquin: 50 ppm	1 ton	94		76		55	99
BHT: 440 ppm	50 kg	123	129	33	37	76	47
	1 ton	187		52		43	140
BHT: 860 ppm	50 kg	146	92	44	34	86	134
	1 ton	131		32		68	94
Natural blend: 390 ppm	50 kg	229	122	65	58	43	119
	1 ton	135		47		62	177
Natural blend: 630 ppm	50 kg	142	130	51	48	58	150
	1 ton	158		62		61	129

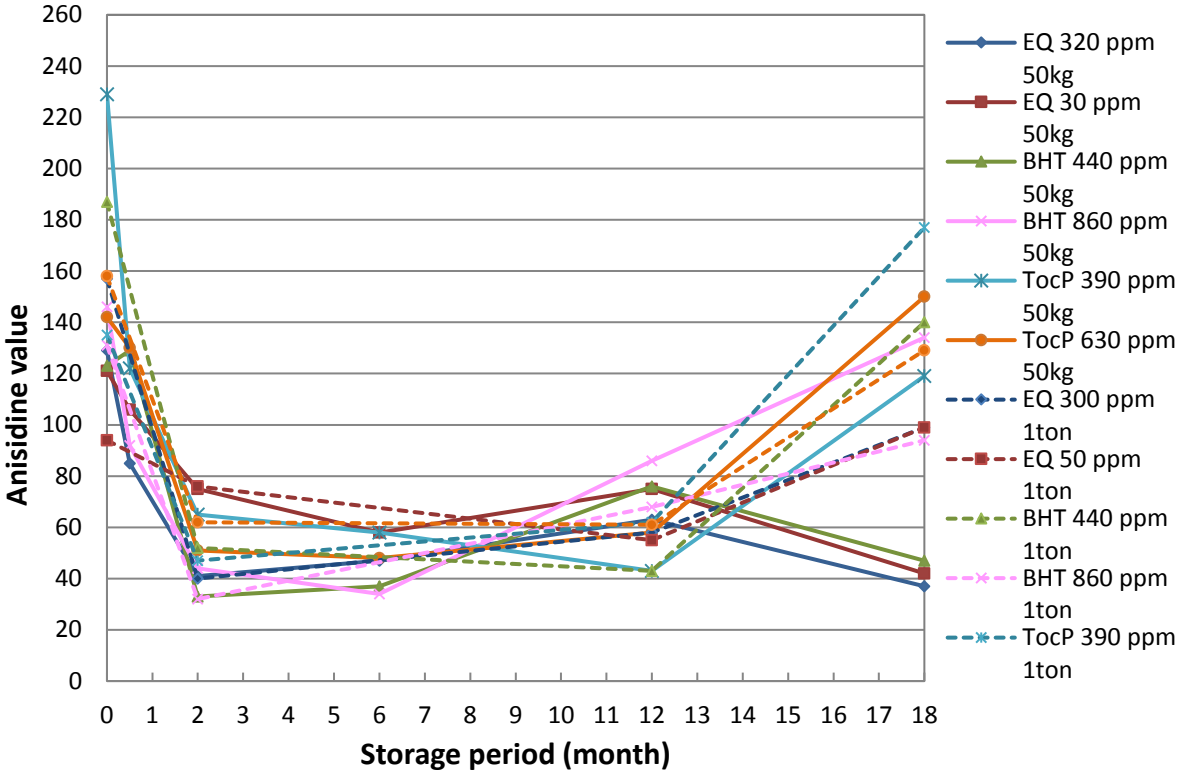

Figure 5: Change in AV in fishmeal of the different antioxidant treatments during 18 months storage.

Total omega-3 fatty acid content

The results of the total omega-3 fatty acid content after storage can be seen in Tables 9, 10 and Figure 6.

The 320/300 ppm ethoxyquin treatments (50kg and 1 ton bags) clearly provided the best protection of fishmeal against oxidation showing negligible decrease of 0.6% and 1.1%, respectively of the omega-3 fatty acids after 12-months and 1.4% and 1.7%, respectively after 18-months of storage period due to oxidation. The 30 ppm (50kg) ethoxyquin treatment showed a marked decrease in the omega-3 content of 51% after 12 months and 67% after 18 months indicating significant oxidation. Dosing fishmeal with 30ppm ethoxyquin clearly does not provide sufficient protection against oxidation. However, the 50 ppm (1 ton) ethoxyquin treatment indicated that even the slightly higher ethoxyquin content along with the bigger storage size bag, resulted in only a 0.6% after 12 months and 10% after 18 months decrease in omega-3 fatty acid content. The efficacy of the 50ppm EQ treatment therefore clearly indicates that a low dosage of 50ppm ethoxyquin does provide sufficient protection against oxidation indicating just how effective ethoxyquin is as antioxidant even at low dosage levels.

The omega-3 fatty acids in the BHT treated fishmeal samples decreased by between 10.3 and 15.3% after 12 months and 15.8 and 20.8% after 18 months storage with little difference shown between the two antioxidant concentrations (440 ppm and 860 ppm) as well as the storage size.

The omega-3 fatty acids in the natural tocopherol blend treated fishmeal samples decreased by between 18.5 % and 28.5% after 12 months and 28.5 – 42.4 % after 18 months with the higher dosage level (630 ppm) showing slightly higher oxidation of the omega-3 fatty acids. High dosage levels of some antioxidants such as tocopherols can act as pro-oxidant and can accelerate oxidation (Rietjens *et al*, 2002). It is therefore important to achieve the optimal dosage level for the best effect. According

to the oxidation levels of the omega-3 fatty acids the natural tocopherol blend is less effective than BHT.

The protection of the valuable omega-3 fatty acids, which in many parts distinguishes fishmeal from other feed ingredients, is very important. Ethoxyquin has proven to be extremely effective at very low dosage levels.

Table 9: The total omega-3 fatty acid content (g/100g) of the different antioxidant treatments during 18 months storage period.

Antioxidant treatment	Storage bag size	Total omega-3 fatty acids (g/100g)					
		DAY	MONTH				
		0	0.5	2	6	12	18
Ethoxyquin: 320 ppm	50 kg	36.1	-	-	-	35.9	35.6
Ethoxyquin: 300 ppm	1 ton	35.8	-	-	-	35.4	35.5
Ethoxyquin: 30 ppm	50kg	35.3	-	-	-	17.3	11.9
Ethoxyquin: 50 ppm	1 ton	34.8	-	-	-	34.6	32.5
BHT: 440 ppm	50 kg	35.6	-	-	-	31.4	30.0
	1 ton	34.0	-	-	-	28.8	30.4
BHT: 860 ppm	50 kg	33.1	-	-	-	29.7	28.6
	1 ton	34.2	-	-	-	30.2	30.0
Natural blend: 390 ppm	50 kg	33.2	-	-	-	25.5	23.5
	1 ton	33.5	-	-	-	27.3	25.8
Natural blend: 630 ppm	50 kg	33.8	-	-	-	24.2	20.8
	1 ton	31.9	-	-	-	22.8	22.6

Table 10: percentage omega-3 fatty acids remaining and decrease after 12 and 18 months of storage.

Antioxidant treatment	Storage bag size	Day 0	12 months		18 months	
		Omega-3 content (g/100g fatty acids)	Remaining % Omega-3 fatty acids (%)	Decrease in omega-3 content (%)	Remaining % Omega-3 fatty acids (%)	Decrease in omega-3 content (%)
Ethoxyquin: 320 ppm	50 kg	36.1	99.4	0.6	98.6	1.4
Ethoxyquin: 300 ppm	1 ton	35.8	98.1	1.9	98.3	1.7
Ethoxyquin: 30 ppm	50 kg	35.3	47.9	52.1	33.0	67.0
Ethoxyquin: 50 ppm	1 ton	34.8	95.8	4.2	90.0	10.0
BHT: 440 ppm	50 kg	35.6	87.0	13.0	83.1	16.9
	1 ton	34.0	79.8	20.2	84.2	15.8
BHT: 860 ppm	50 kg	33.1	82.3	17.7	79.2	20.8
	1 ton	34.2	83.7	16.3	83.1	16.9
Natural blend: 390 ppm	50 kg	33.2	70.6	29.4	65.1	34.9
	1 ton	33.5	75.6	24.4	71.5	28.5
Natural blend: 630 ppm	50 kg	33.8	67.0	33.0	57.6	42.4
	1 ton	31.9	63.2	36.8	62.6	37.4

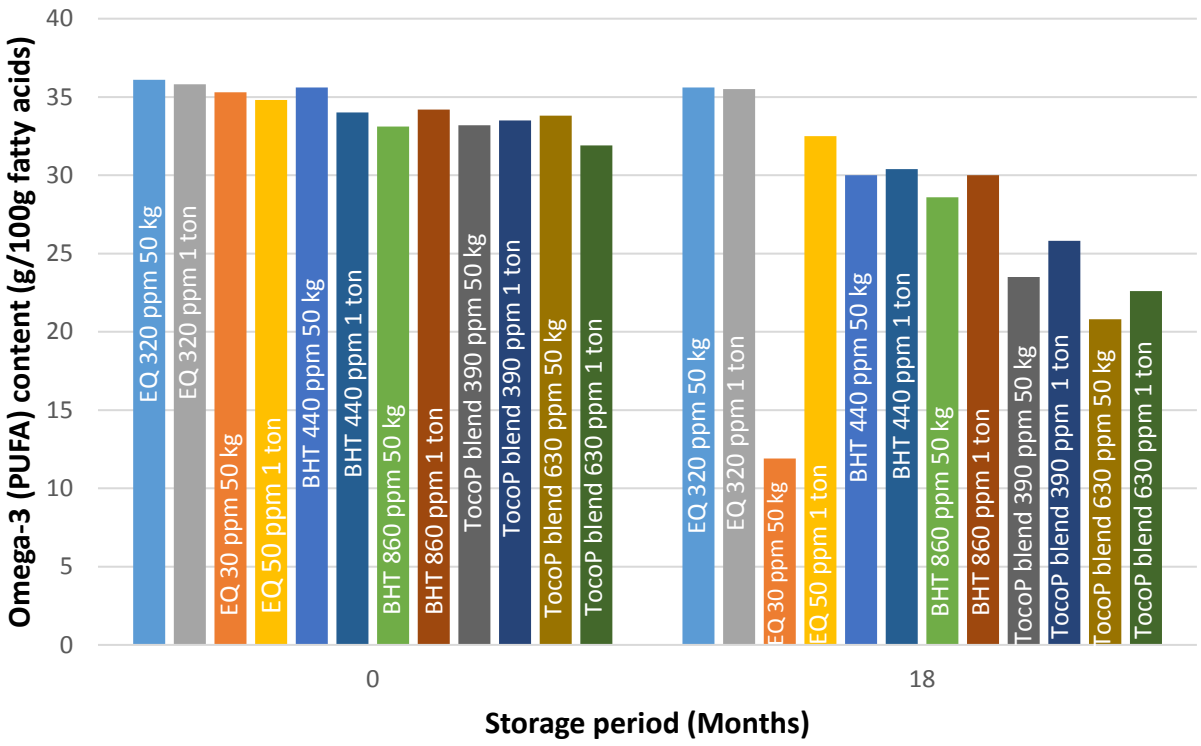

Figure 6: Change in total omega-3 fatty acid content in fishmeal for the different antioxidant treatments at the start of the trial and after 18 months storage.

Temperature readings

The temperature of the fishmeal treatments was taken throughout the storage period along with the ambient temperature and can be seen in Figure 7. The temperature measurement of the fishmeal shows no marked difference between the different antioxidant treatments. The initial temperature of the fishmeal increased to 39°C directly after production but once the antioxidants starts to react with the free radical formed, the temperature decreases and the temperature profile basically followed the ambient temperature. None of the fishmeal treatments increased to a temperature higher than 35°C or higher than roughly 5°C more than the ambient temperature as prescribed in Special Provision 300 (SP 300) by the IMDG which states:

> SP 300 Fish meal, fish scrap and krill meal shall not be transported if the temperature at the time of loading exceeds 35°C or 5°C above the ambient temperature, whichever is higher.

Even the temperature in the fishmeal with the very low dose of 28 ppm ethoxyquin did not overheat during a 12-month storage period and did not increase above unacceptable levels as prescribed by IMDG requirements. There appeared to be a 1°C difference in the 1ton and 50kg bags where the measured temperatures in the 1ton bags are slightly lower. The lower ethoxyquin dosage level, the BHT as well as the natural antioxidant blends all protected fishmeal against overheating and possible combustion.

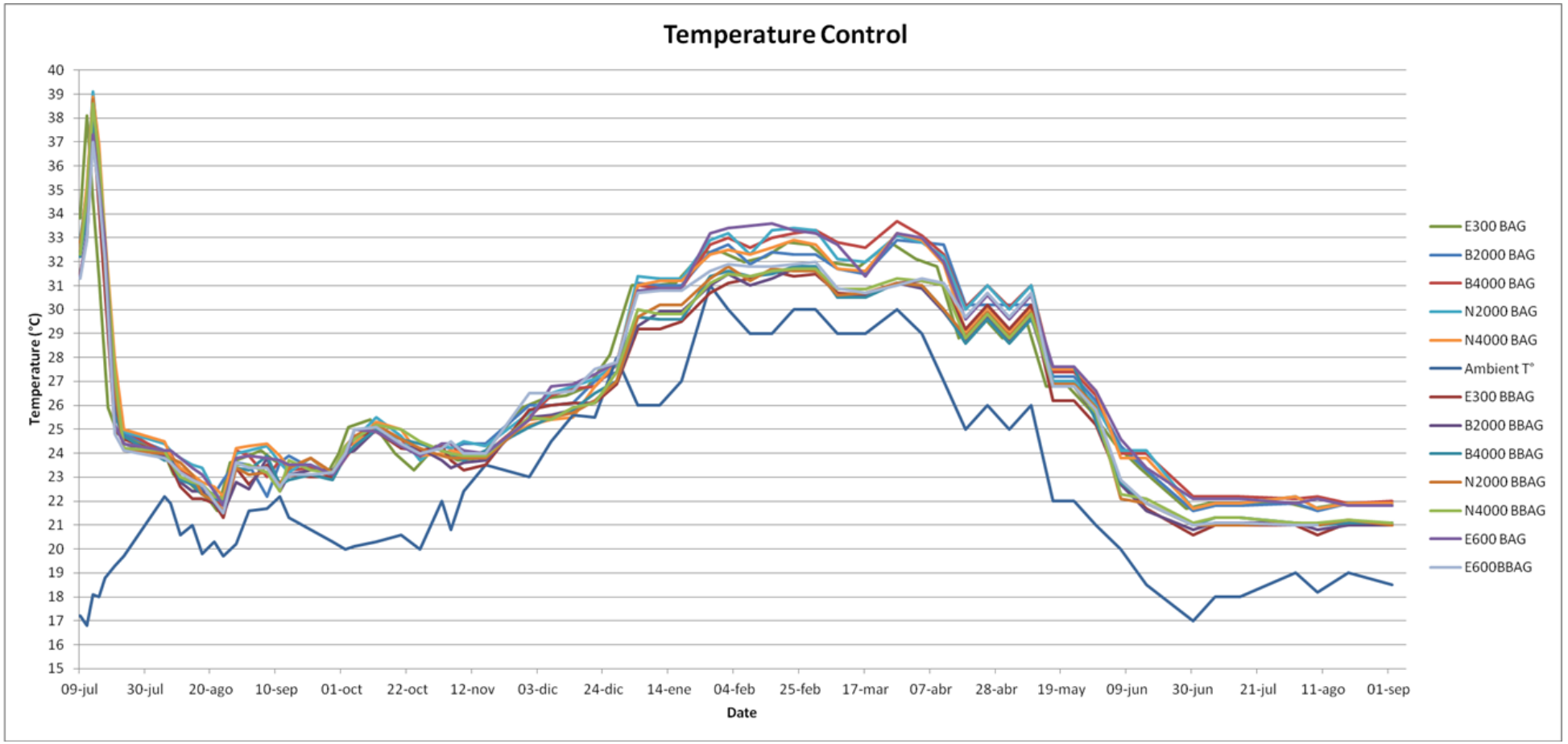

Figure 7: Temperature graph of all the fishmeal treatments along with the ambient temperature. (BAG = 50 kg bag; BBAG = 1 ton bag; E = Ethoxyquin; B = BHT; N = Natural blend)

Self-heating test

The self-heating test is performed by putting fishmeal in a wire basket in an oven at 140°C for a period of 24 hours to determine whether a substance shows self-heating properties (the Schaal Oven Test). If the fishmeal does not combust or reach a temperature higher than 60° above the oven temperature during the 24 hours it passes the test. The results of the self-heating tests can be seen in Table 11. The self-heating test performed on the lowest antioxidant concentration in the 50kg bags (i.e. 300 ppm EQ, 440 ppm BHT and 390 ppm natural tocopherol blend) at 6 months were all negative which indicated that none of the treatments had self-heating properties after 6 months of storage. The higher concentrations were not tested at 6 months because it is expected that if the lower concentration of antioxidant passes the test then the higher concentration would be more stable and should definitely pass the test.

Table 11: Results of the self-heating test after 6 months and 12 months storage.

Antioxidant treatment	**Storage bag size**	**Self-heating test results**	
		MONTH	
		6	**12**
Ethoxyquin: 320 ppm	50 kg	Pass	Pass
Ethoxyquin: 300 ppm	1 ton		Pass
Ethoxyquin: 30 ppm	50kg		Pass
Ethoxyquin: 50 ppm	1 ton		Pass
BHT: 440 ppm	50 kg	Pass	Pass
	1 ton		Pass
BHT: 860 ppm	50 kg		Pass
	1 ton		Pass
Natural blend: 390 ppm	50 kg	Pass	Pass
	1 ton		Pass
Natural blend: 630 ppm	50 kg		Pass
	1 ton		Pass

The 12-month self-heating test was performed on all the treatments. All the treatments passed the test, including the very low ethoxyquin dosage levels of 30ppm (50 kg bag) and 50 ppm (1 ton bag).

The maximum temperature reached during the 24h self-heating test for all 12 treatments can be seen in Figure 8. It is clear that the maximum temperature for each treatment was well below the maximum temperature of 200°C (shown in red dotted line) above which the sample would have failed the test and would therefore be deemed to have self-heating properties. The maximum temperatures reached for the treatments are between 30 - 55°C below 200°C which is well below the 200°C maximum temperature defined as the upper limit in the methodology. Unexpectedly, the 50 ppm (1 ton) ethoxyquin treatment reached the highest temperature of 170°C whereas the 30 ppm (50kg) ethoxyquin treatment reached the lowest temperature of 145°C which was similar to the values obtained by the 390 ppm (50kg) and 630 ppm (50kg) tocopherol treatments of 147.5 and 147.6, respectively. Once again, there appears to be a difference in reaction between samples from 50kg and 1 ton bags where the 1 ton treatments, in general, reached higher temperatures than the 50 kg bag samples.

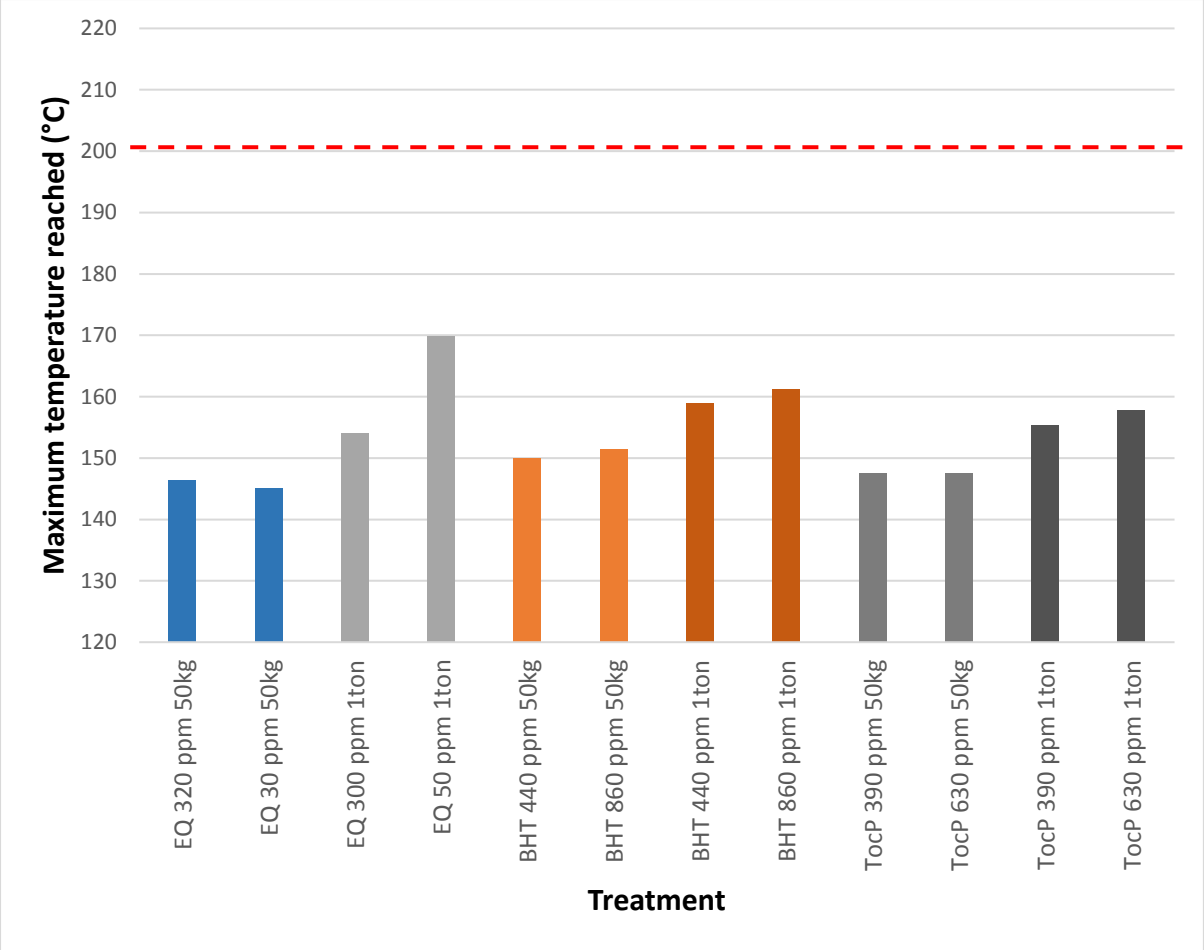

Figure 8: Maximum temperatures reached during self-heating test on all treatments performed at 12 months.

Conclusions

The traditional oxidation quality parameters, FFA, PV and AV could not provide a clear indication of the state of oxidation in fishmeal stored for a long period of time or provide distinct differentiation between the various antioxidant treatments. The omega-3 fatty acid content provided the most reliable indicator of the quality of the fishmeal and also provided a good indication of the efficacies of the various antioxidant treatments.

The results indicated that a 300 ppm ethoxyquin treatment will effectively stabilise fishmeal over a long period of time of at least 18 months as can be seen by the very low rate of oxidation demonstrated by the negligible decrease in omega-3 fatty acid content over a 12-month period. Tocopherols can also be used to stabilise fishmeal as demonstrated by the fact that all the tocopherol treatments passed the self-heating test and the stable temperature of all the treatments during storage. Although there was some protection of the omega-3 fatty acids during the 12-month storage period, the percentage remaining omeg-3 fatty acids was less than for the ethoxyquin or BHT treatments. Data on tocopherols is important for organic feed producers, because in the EU, synthetic antioxidants are not permitted in organic feeds and options for fishmeal stabilisation are therefore very limited.

Ethoxyquin has been shown to be the most efficacious of the available synthetic antioxidants (Blaszcyzyk, A. *et al*, 2013; Lundebye, A-k. *et al*, 2010). The high efficacy of ethoxyquin is not only because of its chemical nature but also due to the fact that its oxidation products also possess strong

antioxidant properties (De Koning, A.J, 2002; Thorrison, S., 1987). Two of its oxidation products, ethoxyquin-dimer and a quinolone have shown to have efficacy values of 69% and 80% of the value of ethoxyquin respectively (De Koning, A.J., 1996).

Even the 50ppm ethoxyquin treatment showed excellent antioxidant activity that was similar, or above, to those of the alternative antioxidants. BHT was slightly more effective than the natural tocopherol blend. The lower dosage levels of the alternatives are more effective than the higher dosage levels where the 390 ppm tocopherol blend provided better protection of the omega-3 fatty acids than the 630ppm tocopherol treatment and similarly the 440ppm BHT provided the same or possibly better protection than the 860 ppm BHT treatment. From the results, it appears that there is a difference in the reaction on storage between the 1 ton bags and the 50kg bags. The difference in reaction could be seen where the 1 ton bags utilises the antioxidants at a slower rate (i.e. more residual antioxidant in the 1 ton bags) than the 50kg bags. In addition, it appears that in general, the 1 ton bags had higher omega-3 fatty acid content remaining after storage than the 50 kg bags which may indicate that that the fishmeal in 1 ton bags deteriorate at a slower rate but this is not an established result and further investigation is necessary.

Ample antioxidant remained after storage, even after 18 months, with sufficient ethoxyquin and BHT remaining to comply with IMO code's requirements at time of shipment of 50 ppm ethoxyquin (new provision) as well as 100 ppm ethoxyquin and/or BHT (current provision) after the 18 months' storage period. The tocopherol residual content of the 630 ppm treatment after 12 months' storage will comply the new proposed shipping amendment to contain at least 250 ppm residual tocopherols at time of shipment. A lower residual level for tocopherols needs further investigation.

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