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NATURAL ANTIOXIDANTS, THE RIGHT CHOICE

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Fats and oils are a major constituent of pet food products. They contribute to their flavor, nutritional intake, texture and palatability. However, lipids are highly sensitive to oxidation during pet food processing or storage, which can lead to rancidity or reducing the food nutritional value. Thus, antioxidants are commonly added to pet food matrices to delay oxidation process. Synthetic antioxidants are widely used to protect oils and fats in pet food industry. Due to the possible adverse effects of synthetic preservatives on pets' health, the consumer's demand for natural antioxidants has tremendously increased in the past decade.

Various plant extracts have been investigated and hundreds of molecules with different chemical structures have been identified as effective natural antioxidants (Brewer, 2011; Pokorny *et al.*, 2001). However, due to availability and economical constraints, only a few of them are currently used in the food industry. Green tea extract, rosemary extract and natural mixed tocopherols have been widely reported for their antioxidative properties in food. Although there are tons of literatures on the activities of these 3 individual natural antioxidants in various matrices, their combined effect to delay oxidation in pet food products has been barely studied.

DIANA Pet Food experts in pet food protection have investigated the antioxidant efficiency and potential synergism of green tea, rosemary and tocopherols when used in pet food.

INTRODUCING THE CANDIDATES

Antioxidants are classified as primary or secondary antioxidants due to their ability to react with free radicals generated during the initiation step of lipid oxidation pathway, to quench $^1O_2$, to chelate oxidation initiators such as metals, or to regenerate free radical scavengers.

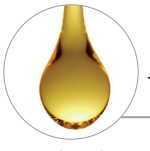
The 3 antioxidants used in this study were all primary antioxidants, each one having different characteristics and mechanisms of action:

Rosemary

Rosemary extract is obtained from leaves of Labiatae (*Rosmarinus officinalis*). Antioxidant properties of this plant extract have been studied since the 1950's. Rosemary extracts are commercially available as water-soluble or oil-soluble liquids or powders. Carnosic acid, carnosol, rosmarinic acid and rosmanol in rosemary extract act as chain breaking radical scavengers to delay lipid oxidation.

Green tea

Of the 3 primary types of tea, green tea (*Camellia sinensis*) has the highest phenolic content. Catechins, extracted from green tea leaves, are the predominant group of green tea polyphenols. Green tea catechins function as effective antioxidants through their scavenging oxygen radicals and chelating metal ions properties.



Tocopherols

Tocopherols are the most commercialized natural antioxidants. They exist in most of vegetable oils and have been extensively studied. Depending on their chemical structure, alpha, beta, gamma and delta tocopherols have different antioxidant activities. These chain breaking antioxidants react with free radicals to delay lipid oxidation.

IMPROVING NATURAL ANTIOXIDANTS COMBINATIONS USING EXPERIMENTAL DESIGNS

Determining the perfect combination of 3 natural antioxidants is not easy since the dosage effect of each antioxidant has an influence on the overall performance of the blend. Thanks to the surface response methodology, it is possible to determine the contribution of each antioxidant to the performance of their combination.

Different dosages of green tea extract, rosemary extract and natural mixed tocopherols were mixed in sunflower oil or in poultry fat according to 2 separated central composite designs for each fat. Antioxidative performance of 17 blends was evaluated by Rancimat. In this method, oxidation was forced by air bubbling and heating at 110°C for poultry fat and 90°C for sunflower oil. Induction time of oxidation was measured. The efficiency of the antioxidants combinations was determined based on their ability to delay oxidation: the higher induction time, the better. A synthetic antioxidants mix made of BHA, propylgallate and citric acid was added to the experiment as a reference.
Linear, quadratic and interaction effects of antioxidants were then analyzed using a general linear model on Statgraphics. This model establishes a rank between effects of each factor on the oxidation in order to select the best one depending on the fat. It allows predicting the optimal dosage of each antioxidant.

THE SOLUTION = SEVERAL SOLUTIONS!

As expected, antioxidative efficiency of the blends were different according to the nature of the fat. Table I shows the statistical contribution of each antioxidant and their interactions in both fats.

Table 1: Statistical analysis of the effect of individual antioxidant and their interactions on induction time (in red, p value < 0.05).
RO = Rosemary; GT = Green tea; TO = Tocopherols.

Effect	p value	
	poultry fat	sunflower oil
Rosemary	0	0.355
Green tea	0.09	0.003
Tocopherols	0.001	0.122
Rosemary²	0.027	0.792
Green tea²	0	0.038
Tocopherols²	0.331	0.48
RO*GT	0.314	0.716
RO*TO	0.809	0.2
GT*TO	0.471	0.874

> In **poultry fat**, all 3 extracts significantly contributed to delay oxidation (Table I). Interactions between compounds were not significant at p = 0.05, showing an additional but not synergistic effect of the natural extracts.

Figure 1 shows that the longest induction times (in red) could be obtained with various dosages of the 3 antioxidants. Dose effects of rosemary and green tea were not linear but quadratic as seen on the curved dispersion of the red area. Surface response methodology showed an optimal protection of poultry fat when a mixture containing 25% green tea, 11% rosemary and 64% mixed tocopherols was added.

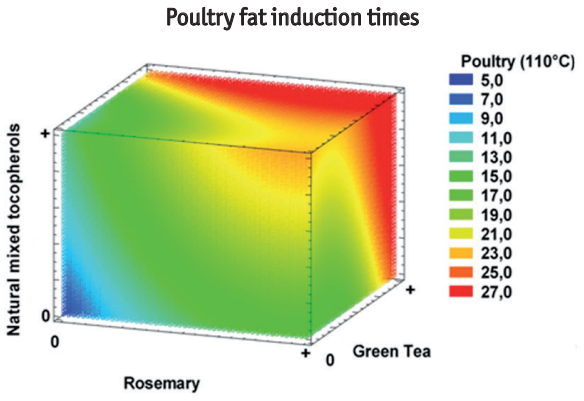


Figure 1: Induction times (hours Rancimat) for poultry fat containing mixtures of green tea, rosemary extract and mixed tocopherols.

> **Sunflower** oil was not well protected by rosemary and tocopherols, but clear benefit was seen when green tea was added to the formulation (Table I). Dose effect of this antioxidant was both linear and quadratic. In the framework of this experiment, the optimal amount of green tea was 31% in the blend (Figure 2).

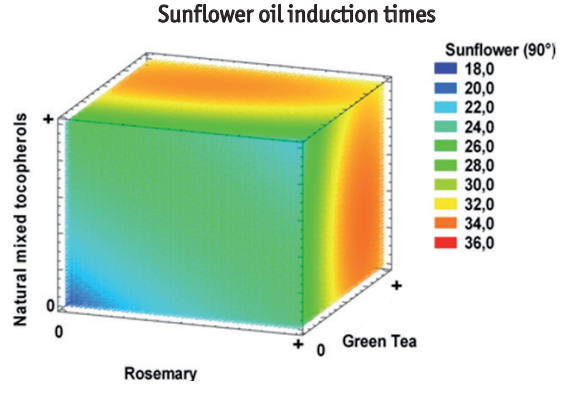


Figure 2: Induction times (hours Rancimat) for poultry fat and sunflower oil containing mixtures of green tea, rosemary extract and mixed tocopherols.

ON THE INTEREST OF COMBINING ANTIOXIDANTS

In order to compare the efficiency of the antioxidants mix used in both fats, a "protection factor" was calculated based on Rancimat results. This indicator was defined as the difference between the induction time of the antioxidized oil and the induction time of the same oil without antioxidant. The higher protection factor, the better.

Figure 3 shows, for each fat, the protection factors of:
- the 3 natural antioxidants used alone (rosemary, green tea, tocopherols)
- an optimal mix of the 3 antioxidants (Greenway™ 3D)
- a synthetic mix made of BHA, propylgallate and citric acid (synthetic).

In both fats, rosemary extract, green tea or tocopherols gave a limited protection when used alone. However, blending these natural compounds allowed a similar or even better protection of lipids than a synthetic antioxidant solution.

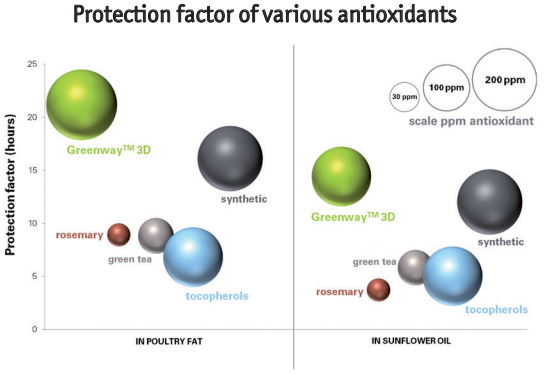


Figure 3: Protection factor of antioxidants according to fat nature

Several studies have already demonstrated the cooperative effects of antioxidants to delay oxidation in vegetable or fish oils-based model systems. However, conclusions may be different depending on the media in which antioxidants are applied. For example, Yin (2012) reported a synergistic or an additive effect between green tea extract and α-tocopherols in sunflower oil or in emulsions. However, other studies demonstrated an antagonism between those 2 natural antioxidants. Bulk oil and water-in-oil or oil-in-water emulsions behave differently under similar oxidation conditions. In the present study, combinations of green tea, rosemary and natural mixed tocopherols showed different antioxidative efficiency depending on the type of lipid base. This can be explained by the fact that the antioxidant activity does not only depend on the chemical reaction between an antioxidant molecule and lipids but also on the environment of the reaction. Water content, viscosity, temperature or porosity of matrices highly influence the diffusion of antioxidant molecules in food products and thus affect the antioxidant performance.

WHAT ABOUT KIBBLE PROTECTION?

In pet food industry, antioxidants are commonly added to raw materials in order to protect lipid from oxidation during the kibble manufacturing process. Furthermore, an additional antioxidant application through fat coating after extrusion is generally done to ensure lipid preservation during the kibble shelf-life.

A complementary study was conducted to assess the antioxidative efficiency of a blend of natural mixed tocopherols and rosemary extract (Greenway L3™) when coated on kibbles with poultry fat. Accelerated storage of coated kibbles was performed at 50°C. The oxidative status of kibbles was evaluated by measuring the levels of hexanal, tocopherols and carnosic acid on a weekly basis. Hexanal, a secondary oxidation product, is indeed one of the most representative volatiles released during oxidation.

Thanks to the use of a combination of natural mixed tocopherols and rosemary extracts, hexanal release in coated kibbles was lower than in non antioxidized kibbles (Figure 4). Levels of other volatiles such as octanal, 2-hexenal and hexanoic acid were also decreased by the addition of the natural antioxidants.

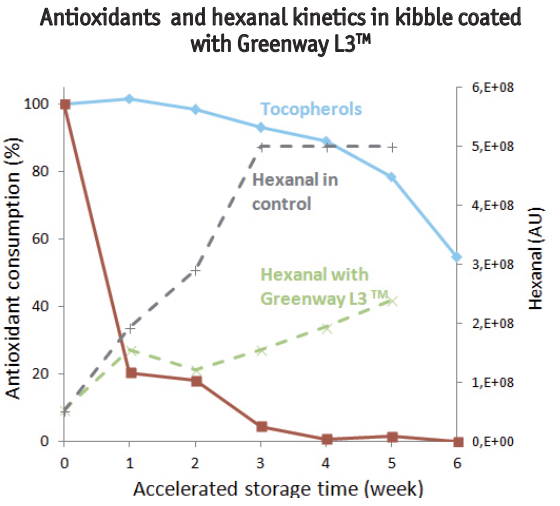


Figure 4: Antioxidant consumption and hexanal release (arbitrary unit) during accelerated storage at 50°C for control kibbles without antioxidant and kibbles antioxidized with a combination of natural mixed tocopherols and rosemary extract (Greenway L3™).

In parallel with the release of secondary oxidation products, a consumption of natural antioxidants was also observed (Figure 4). Levels of tocopherols and carnosic acid from rosemary extract in kibbles decreased during the accelerated storage, as antioxidants reacted with free radicals from lipid oxidation. It is very interesting to notice that carnosic acid was consumed before tocopherols: carnosic acid first reacted with free radicals and in a second step, when carnosic acid level became very low, tocopherols were used to prevent lipid from oxidation. The additional antioxidative effect of tocopherols and rosemary observed previously in poultry fat could also be seen on kibbles. These antioxidants consumption kinetics might be explained by the ability of carnosic acid to regenerate tocopherols (Pokorny *et al.*, 2001), or by its increased affinity with free radicals compared to tocopherols.

CONCLUSION

Natural antioxidants can successfully be used to protect pet food. When selected carefully, they can perform even better than synthetic antioxidants.

The comprehensive study conducted by DIANA Pet food demonstrated that using green tea extract, rosemary extract and natural mixed tocopherols constitute a promising solution to naturally protect both fat and kibbles. If each antioxidant showed limited efficiency against oxidation when used alone, blending them allowed reaching a high level of protection.

However, there is not one universal solution to protect pet food. Indeed, antioxidants do not behave similarly according to the matrix to which they are added. Different combinations of natural antioxidants have to be designed specifically for each targeted matrix. The use of appropriate experimental design and surface response methodology can allow determining the optimal dosage of each compound in the blend according to the final application.

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IF YOU NEED FURTHER INFORMATION, DO NOT HESITATE TO CONTACT THE AUTHOR

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