The art of formulations:
5 keys to selecting the best botanical extract

Margarita Altable PhD. NBD Technician. Oxiris Chemicals

The growing interest in food safety by consumers and the rigorous evaluation of additives by the **European Food Safety Authority** have led to the investigation of more natural alternatives such as **botanical extracts** for incorporation into food formulations.

Undoubtedly, **natural resources** provide a **wide range of ingredients** of great interest for the **animal food** sector, and ensuring their properties remain intact until the moment of their application is an essential requirement.

However, the legalization of new additives is a tedious process that requires a series of **analytical**, **efficacy**, **stability** and **toxicological** studies which guarantee correct and safe use.

Synthetic and natural antioxidants

In the specific case of **antioxidants,** a kind of additives which allow the extension of the life span of food, the list of authorised substances is very limited. It includes synthetic antioxidants, such as **BHT** (E-321), **BHA** (E-320) and **propyl gallate** (E-310), and natural oxidants also, such as **ascorbic acid** (E-300) and the **extract of tocopherol** (E-306).

Both groups have certain characteristics which make them attractive for certain applications. In any case, it is desirable to evaluate each case independently and thereby to select the **antioxidant** or **mixture of antioxidants** which best suit the needs of the producer.

Synthetic antioxidants	Natural antioxidants
More economical	Better acceptance for consumers
Medium-high antioxidant activity	Soluble in water: ascorbic acid
Soluble in oils and fats: BHT and BHA	Soluble in oils and fats: tocopherol
Hardly provide colour, odour and taste	High thermal stability
Chemical production	Derived from natural resources

Table 1. Main characteristics of synthetic and natural antioxidants.

What are botanical extracts?

The so-called botanical extracts are substances obtained from some parts of a plant (roots, stems, leaves) which present beneficial bioactive properties for organisms. For this reason, it is possible to include them in diets as a **nutritional additive** and/or acting in the food as **a technological additive**.

OXIRIS
Extending life

Ctra. C-35, Km 59,
Pol. Ind. Nord Est
08470 Sant Celoni,
Barcelona, Spain
Phone nº: +34 93 867 49 97
expert@oxirischemicals.com
www.oxirischemicals.com

These extracts are obtained from **vegetable resources** through different methodologies such as pressing, distillation, extraction with solvents or supercritical fluids or a combination of them all.

The method used and the physical-chemical conditions used determine the quality of the final extract, affecting parameters such as the concentration of the **main active ingredient** or the presence of undesirable substances which may come from the extract itself.

Due to the numerous variables associated with obtaining botanical extracts, the producers face a series of challenges with a great impact on the **quality**, **effectiveness** and **cost** of the extract, such as:

- → the **optimisation** of farming and technological extraction processes
- → the ability to **reproduce** the composition, quality, and power of the extracts (standardization)
- → **toxicological safety** of all compounds of the extracts and their metabolites

5 keys to selecting botanical extracts

Bearing this in mind, at the moment of evaluating the use of botanical extracts in our formulations, antioxidant producers, nutritionists, and feed and supplement producers should consider **five basic factors** related to the extracts, just as **EFSA** recommends for other applications (EFSA Journal 2009; 7(9):1249); (EFSA Journal 2014;12(3):3593):

Origin:
Knowing the relevant information about:

- → the **natural resources** used (the species, the geographic origin, the cultivation method).
- → whether **genetically modified seeds** are used.
- → the method of extraction and purification used on the extract and the processes conditions (**temperature**/**solvent**).

In the case of the **synthetic homologue**, it is important to consider the conditions of the production process and other factors that can affect the efficiency of the additive. Some factors are the presence of associated impurities or the differences both in the physical-chemical properties (e.g. the presence of isomers or the stability) and the **activity performance** with respect to the active ingredient of natural origin.

Chemical composition:
Unequivocal **identification** of the active ingredients of the extract and, if possible, of the other components or at least of those most relevant.

Activity:
Quantification of the **activity** of the extract to ensure its correct dosage.

Stability:
Knowing the conditions under which the physical-chemical and metabolic **degradation** of the components of the extracts occur, and identifying the compounds produced.

Safety:
Confirming the **absence of negative effects** caused by any of the components of the extract, metabolites or degradation products in the organism.

OXIRIS
Extending life

Ctra. C-35, Km 59,
Pol. Ind. Nord Est
08470 Sant Celoni,
Barcelona, Spain
Phone nº: +34 93 867 49 97
expert@oxirischemicals.com
www.oxirischemicals.com

In the case of some botanical extracts which are used traditionally in nutrition, such as the extract of tocopherol, extensive and established knowledge based on experience is available. Nevertheless, it is different regarding new extracts or those in which modifications have been made in the production method.

Therefore, it is necessary to perform new studies which confirm their activity and toxicological safety.

Extract of tocopherol: the natural antioxidant

Tocopherol has been selected by **Oxiris** as an ingredient in some formulas (**NATUROL**) because it is one of the most relevant ingredients in nutrition due to its antioxidant activity in food and its activity as vitamin E inside organisms.

In fact, tocopherol is a **group of organic compounds** formed by a chromanol ring which is connected to a hydrocarbon chain formed of 16 carbon atoms. Some methyl groups can be located in different positions of the phenolic ring, giving rise to four different isomers: **alpha, beta, delta** and **gamma.**

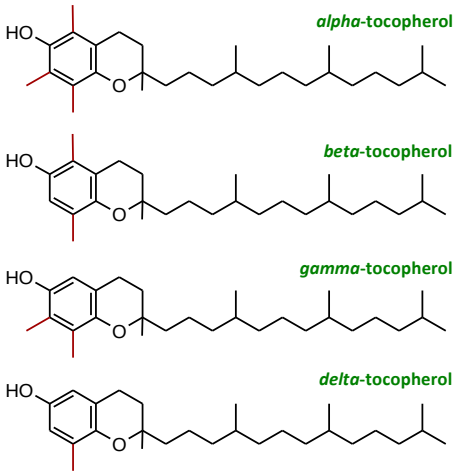


Image 1. Molecular structure of the four isomers of tocopherol.

Regarding its origin, tocopherols can be found in **vegetable oils** such as soya, corn, wheat and palm oil. Tocopherols are isolated from these oils, concentrated, and purified to separate them from other substances such as sterols or fatty acids. Tocopherol extracts are commercialized as **mixtures of antioxidants** (often diluted with soya or sunflower oil) with concentrations of total tocopherols between **30-80%** and a **variable composition of isomers**.

Regarding the antioxidant capacity of tocopherols, diverse studies conclude that these isomers do not have the same activity and that this changes in function of parameters such as **temperature** or the **medium** which they are dissolved in. These conditions should be considered at the moment of application.

37ºC:	alpha > beta > gamma > delta (antioxidant activity similar to the biological as vitamin)
50-100ºC	delta > gamma > beta > alpha

Table 2. Antioxidant activity of the four isomers of tocopherol at different temperatures (Food Antioxidants, CRC, pag 180).

OXIRIS
Extending life

Ctra. C-35, Km 59,
Pol. Ind. Nord Est
08470 Sant Celoni,
Barcelona, Spain
Phone nº: +34 93 867 49 97
expert@oxirischemicals.com
www.oxirischemicals.com

On the other hand, **alpha-tocopherol** is the isomer with the most activity as **vitamin E** (twice that of beta and gamma and 100 times more than delta) but only one of its eight possible stereoisomers are present in natural resources. This stereoisomer, the denominated **RRR-alpha-tocopherol** or d-configuration, is the only one classified and commercialized as vitamin E. It is often traded as the most stable form, tocopheryl acetate (Food Antioxidants, CRC, pag 180).

Chemical synthesis is an alternative to the extraction of tocopherols from plant sources that allows each isomer to be obtained independently. For example, alpha-tocopherol is synthesized by the reaction of 2,3,5-trimethylhydroquinone with phytol, isophytol or phytyl halide at the presence of an acid. When this isomer is chemically obtained the **eight stereoisomers** (RRR, RRS, RSR, RSS, SSS, SRS, SRR, SSR) are formed in a similar proportion. This mixture of stereoisomers is called **all-rac-alpha-tocopherol** and it is authorised as both an **antioxidant** and **vitamin E**.

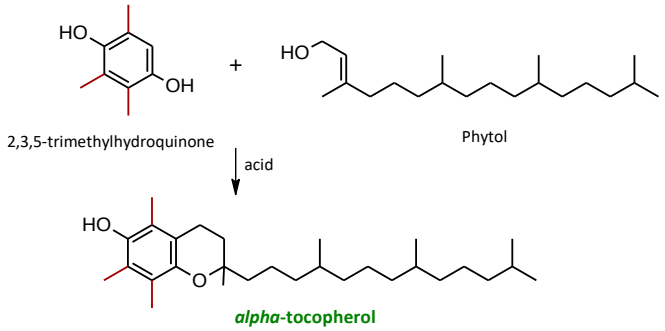


Image 2. Chemical synthesis route of alpha-tocopherol (all-rac-alpha-tocopherol).

Feed code	Classification	Substance	Origin
1b306 (i)	Antioxidant	Extract of tocopherol (Mixture of isomers)	Natural
1b306 (ii)	Antioxidant	Extract enriched with tocopherol (Enriched with delta)	Natural
1b307	Antioxidant	alpha-tocopherol (synthesis)	Synthetic
3a700	Vitamin E	all-rac-alpha-tocopheryl acetate	Synthetic
		RRR-alpha-tocopherol	Natural
		RRR-alpha-tocopheryl acetate	Semisynthetic

Table 3. List of tocopherols authorised in Feed as antioxidants and vitamin E Regulation (EC) No 1831/2003. European Union Register of Feed Additives. Edition 05/2021 (293). Annex I – 19.08.2021.

Analysis and control

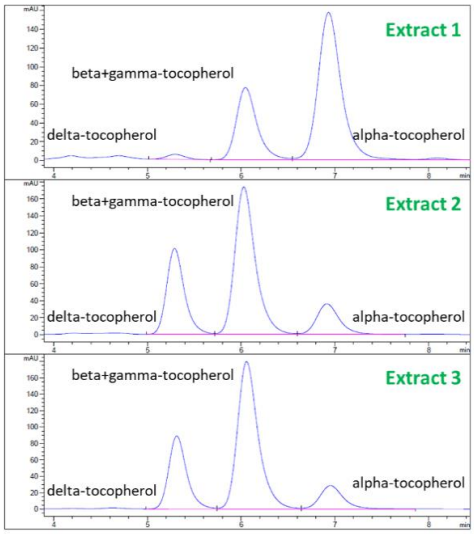
For all these reasons, it is essential for manufacturers of antioxidant mixtures to **identify** and **quantify** the different isomers of tocopherol, as well as analysing their antioxidant activity, in order to select the most appropriate extract.

When comparing the antioxidant activity of different extracts, it is possible that their performance is different. This can happen for various reasons: different content of total tocopherols, non-homogeneous extracts, different isomeric profiles or synergistic effects with other components of the extract.

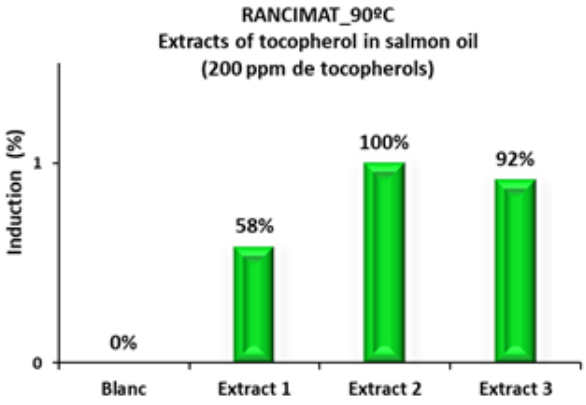
OXIRIS
Extending life

Ctra. C-35, Km 59,
Pol. Ind. Nord Est
08470 Sant Celoni,
Barcelona, Spain
Phone nº: +34 93 867 49 97
expert@oxirischemicals.com
www.oxirischemicals.com

In Graphic 1, a comparative study of antioxidant activity of three extracts with similar **content of total tocopherol** (15%) is shown. Those extracts with the greater proportion of delta and gamma isomers are those with the best performance in **Rancimat** experiment (Graphic 2).



Graphic 1. Comparative study of the isomeric profile (HPLC) of three extracts of tocopherol with a total content of tocopherols of 15%.



Graphic 2. Comparative study of the antioxidant activity (Rancimat) of three extracts of tocopherol with a total content of tocopherols of 15%.

In conclusion, although botanical extracts are excellent allies in the challenges facing the food sector, knowing the **origin, composition, activity, stability** and **safety** are five keys which help us to guarantee an optimal performance of our formulas while maintaining safety standards.

OXIRIS
Extending life

Ctra. C-35, Km 59,
Pol. Ind. Nord Est
08470 Sant Celoni,
Barcelona, Spain
Phone nº: +34 93 867 49 97
expert@oxirischemicals.com
www.oxirischemicals.com