Metrohm

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Determination of the antioxidant activity by the Rancimat method

Branch

Food, stimulants, beverages, flavors; cosmetics; pharmaceutical industry

Keywords

Oxidative stability; oxidation stability; rancidity; 892; oils; fats; antioxidants; antioxidant activity; antioxidative activity index; branch 4; branch 7; branch 12

Summary

Antioxidants have gained a great importance in stabilizing foodstuffs, cosmetics, and pharmaceuticals by suppressing oxidation of fats and oils and other components in the respective products. Whereas in foodstuffs and cosmetics mainly the formation of rancid flavors has to be avoided, it is important in medicines to protect the active ingredients from being decomposed.

The antioxidant activity of antioxidants can be investigated using the Rancimat method. Therefore, the antioxidant of interest is mixed with pure lard, which is the reference. This mixture is heated to precisely 110 °C while a constant stream of air flows through the sample. The lard is oxidized during this process and the decomposition products are transferred into a measuring cell. Therein, some of the oxidation products like low-molecular-weight organic acids increase the electrical conductivity, which serves as a measure for the oxidation progress. A sharp increase in the conductivity indicates the point where the sample is oxidized at a fast rate. The time after which this process starts is defined as the induction time. To test different antioxidants, pure lard is used as the reference. The antioxidant activity can be measured by the antioxidative activity index which is defined as the induction time of the reference with additive divided by the induction time of pure lard. It characterizes the efficacy of a certain antioxidant. This method can help to choose an antioxidant for a specific product.

Sample

Antioxidants: (±)-α-tocopherol, gallic acid, 6-O-palmitoyl-L-ascorbic acid, butylated hydroxytoluene, butylated hydroxyanisole

Reference/control: Pure lard

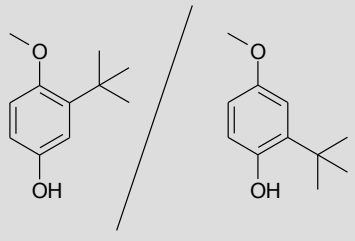
Instruments

892 Professional Rancimat	2.892.0010
Equipment for determination of temperature correction	6.5616.100
StabNet PC software	6.6068.xxx
Auxiliary instruments for sample preparation • Thermostat bath (or oven) • Mortar and pestle • Laboratory balance (resolution ± 0.1 mg)	

Reagents

- Deionized water (ISO 3696 Type II)
- Pure lard
- Antioxidants:

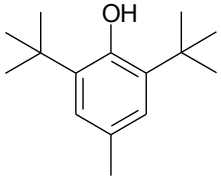
2(3)-*tert*-Butyl-4-hydroxyanisole (butylated hydroxyanisole, BHA, isomeric mixture), 98.5%, CAS 25013-16-5



~90% ~10%

M = 180.25 g/mol

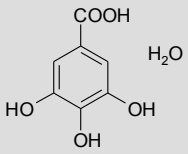
2,6-Di-tert-*butyl*-4-methylphenol (butylated hydroxytoluene, BHT), 99.0%, CAS 128-37-0



M = 220.36 g/mol

Gallic acid monohydrate

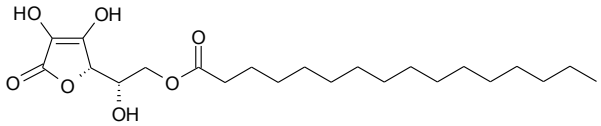
$C_7H_6O_5 \cdot H_2O$, 99%, CAS 5995-86-8



M = 170.15 g/mol

6-O-Palmitoyl-L-ascorbic acid (Palmitoyl ascorbate)

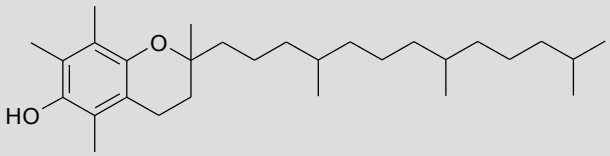
$C_{22}H_{38}O_7$, 99%, CAS 137-66-6



M = 414.54 g/mol

(±)-α-Tocopherol (all-racemic mixture)

$C_{29}H_{50}O_2$, CAS 10191-41-0



M = 430.72 g/mol

Sample preparation

20 g of pure lard is heated to 50 °C in a closed flask in a thermostat bath for 30 min to melt. Then, 4 mg of the antioxidant are added to the lard and the mixture is stirred well with a glass rod to get a homogeneous solution. The same melting procedure was used for the pure lard as well.

Analysis

Preparation of the Rancimat

The heating block is heated up to the respective temperature.

Preparation of the measuring vessel

The measuring vessel is filled with 60 mL deionized water and placed on the Rancimat together with the measuring vessel cover.

Preparation of the reaction vessel

For each determination, a new reaction vessel is used. To remove particles (e.g., from the cardboard box), the reaction vessel is air-cleaned inside and outside by a sharp stream of nitrogen. Then, 3.0 ± 0.1 g of lard or antioxidant/lard mixture are weighed directly into the reaction vessel without contaminating the side walls. The reaction vessel is closed with a reaction vessel cover assembled with an air inlet tube.

Determination

Before the determination can be started, the temperature of the heating block has to be stable. The two tubings between Rancimat and reaction vessel and between reaction vessel and measuring vessel are connected. Then, the reaction vessel is placed in the heating block and the measurement is started immediately.

Parameters

Sample size	3.0 g ± 0.1 g
Measuring solution	60 mL deionized water
Gas flow	20 L/h
Temperature	110 °C
Temperature correction	Automatic
Stop criteria	Endpoints Conductivity: 100 µS/cm
Evaluation	Induction time automatic
Evaluation sensitivity	1.0

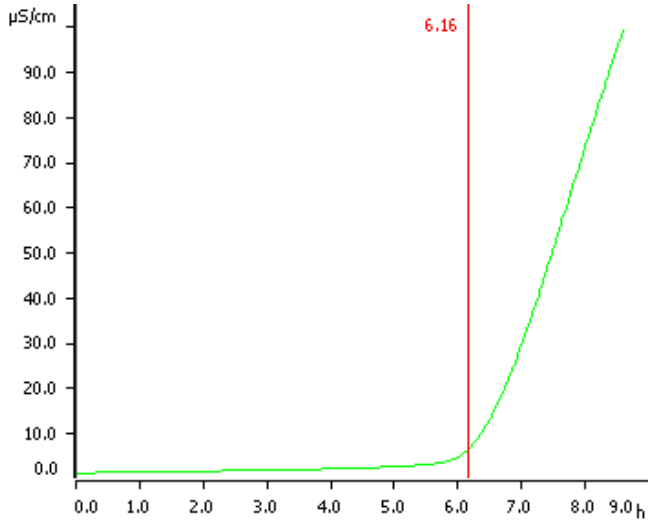
Calculation

The antioxidative activity index (AI) is defined as

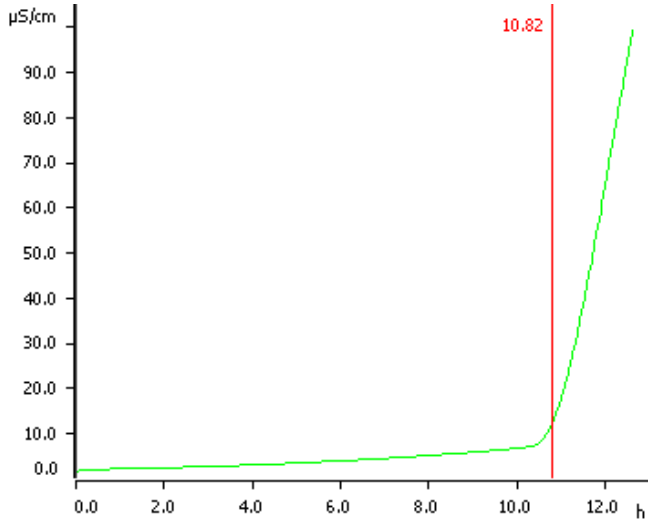
$$AI = \frac{\text{Induction time with additive}}{\text{Induction time without additive}}$$

Example determinations

BHT



α-Tocopherol

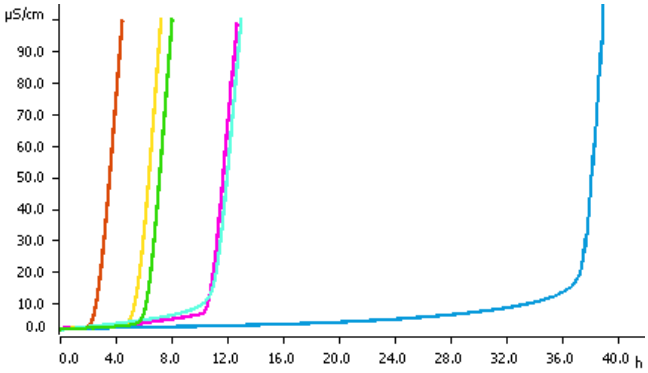


Results

Antioxidant	Induction time / h	AI
Gallic acid	37.84	15.2
(±)-α-Tocopherol	10.83	4.4
Palmitoyl ascorbate	5.05	2.0
BHT	6.18	2.5
BHA	10.92	4.4
Control	2.49	

The results are the mean values of four determinations each.

The curves are displayed in the following figure.



Color	Sample
	Control
	Palmitoyl ascorbate
	BHT
	α-Tocopherol
	BHA
	Gallic acid

For improved visibility, only one curve per substance is displayed.

Some more antioxidants are shown in the following table. The values have been obtained from literature (see the references section). These antioxidants are mainly isolated compounds from plant extracts such as from green tea, rosemary, and red sage. These plants are commonly used in traditional Chinese medicine.

Antioxidant	Induction time / h	AI
Epigallocatechin gallate	28.80	13.40
Epigallocatechin	26.50	12.32
Epicatechin gallate	15.80	7.35
Epicatechin	5.30	2.46
Carnosol	25.40	9.63
Carnosic acid	30.60	14.23
Ursolic acid	2.47	1.15
Tanshen I	8.93	4.15
Dihydrotanshinone	10.04	4.67
Tanshinone IIA	2.43	1.13
Tanshinone IIB	5.45	2.53
Danshenxinkun B	4.38	2.04
Control	2.15	

Detailed results

Gallic acid

Sample	Induction time / h
1	37.69
2	38.14
3	38.44
4	37.09
Mean	37.84
RSD	1.3%

α-Tocopherol

Sample	Induction time / h
1	11.03
2	10.82
3	10.78
4	10.70
Mean	10.83
RSD	1.1%

Palmitoyl ascorbate

Sample	Induction time / h
1	5.02
2	5.34
3	4.63
4	5.15
Mean	5.04
RSD	5.2%

BHT

Sample	Induction time / h
1	6.12
2	6.16
3	6.10
4	6.35
Mean	6.18
RSD	1.6%

BHA

Sample	Induction time / h
1	10.01
2	11.35
3	11.14
4	11.18
Mean	10.92
RSD	4.8%

Control

Sample	Induction time / h
1	2.39
2	2.53
3	2.52
4	2.53
Mean	2.55
RSD	3.1%

Comments

- The antioxidative activity index can serve as a means to compare different antioxidants. Anyhow it has to be considered that for practical reasons the addition of antioxidant is specified as its mass fraction in the reference and the molecular weight is neglected. Therefore, the antioxidative activity index can only be used to compare substances according to their mass fraction in the antioxidant/lard mixture and is no means for the potency of the substance itself.
- The melting step does not have an influence on the induction time of pure lard. This was tested with pure lard that was not heated before the measurement. Without melting, pure lard showed an induction time of 2.55 h (mean of 4 samples, standard deviation = 0.08 h), which was not significantly higher than with melting (2.49 h, standard deviation = 0.06 h).
- The melting step can also be carried out in a closed bottle in a heating oven. In this case, 20 g of pure lard are weighed in a well-cleaned glass bottle and closed with a screw cap. The glass bottle is put in the heating oven for 30 min at 50 °C. Thereafter, the lard is a transparent liquid which can easily be mixed with the antioxidant. This melting procedure also does not have an influence on the induction time. 30 min is the maximum time for lard to be liquefied at 50 °C. With longer pretreatment or at higher temperatures, the induction time is decreased with respect to the untreated control sample.

- When lard and antioxidants are mixed, care has to be taken that the antioxidant is mixed homogeneously. Especially if the antioxidant does not dissolve quickly in lard at 50 °C the antioxidant grain size distribution has to be very narrow. The simplest way to ensure this is to pulverize the antioxidant, for example, with a mortar and pestle if it is crystalline. Then, it can be mixed with lard very homogeneously.
- The induction time of the reference (pure lard) should be determined frequently, i.e., once per week. Also care has to be taken that the lard from the sample/lard mixture is always from the same batch (or package) as the pure lard reference, since the induction time might be different between batches.
- Pure lard should always be used fresh and stored in a refrigerator at approx. 5 °C.
- In this document, the application of pure lard as the control substance is described. Animal fats naturally contain no antioxidants. Therefore, they can be used to study the effect of an antioxidant alone. Vegetable oils usually contain natural antioxidants, which can have an impact on the activity of the added antioxidant. Therefore, the activity of a pure antioxidant can't be determined with a vegetable oil. Nevertheless, a product such as sunflower oil can be tested together with different antioxidants and concentrations using this approach to find a suitable combination for enhanced stability.

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