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The Reduction of Oxidation of Food Products Using Dioxygenases

Liesbeth C. M. Bouwens* and Yvonne E. Bruggeman

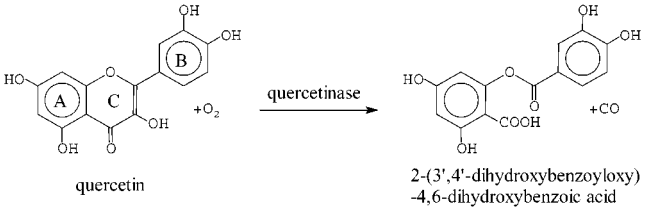
Unilever Research and Development, Vlaardingen, The Netherlands

Abstract:

A novel way of deoxygenating food products by oxygen scavenging enzymes has been developed, especially suitable for food products comprising mono or polyunsaturated fatty acids or both which are very sensitive to oxidation. It has been found that dioxygenases, which are enzymes capable of oxidising substrates by inserting one or two oxygen atoms into the substrate using molecular oxygen, are very effective in oxygen removal in food products. Moreover, no reactive species are formed during this reaction. Furthermore, the substrates for dioxygenases are antioxidants, which have the advantage that a combination-effect can be achieved, particularly because the substrate retains its antioxidant activity after being oxidised. The use of quercetinase and catechinase, both dioxygenases, as oxygen-scavenging enzymes for off-flavour prevention in oil-in-water (o/w) emulsions was found to be highly efficient, and the oxygen concentration could be reduced to zero. The enzymes quercetinase and catechinase were able to deoxygenate o/w emulsion in the presence of the antioxidants quercetin and catechin, respectively. Especially the combination of quercetinase and quercetin resulted in a very effective off-flavour reduction of 80−97% as determined by GC analysis of 7 out of 10 volatiles labeled as off-flavour.

Introduction

Food products often contain mono or polyunsaturated fatty acids or both which are very sensitive to oxidation. Oxygen is one of the origins of product deterioration due to oxidation. Polyunsaturated fatty acids (PUFAs) are particularly sensitive to oxidation when applied in emulsions. Options to solve this problem are: (1) the reduction of oxygen concentration, (2) addition of antioxidants, (3) lowering storage temperature, and (4) scavenging of metal ions (mainly Fe, Cu) with sequestrants. We have found two unique enzymes (dioxygenases) that are active to consume oxygen in oil-in-water emulsions.[1] Moreover, these enzymes can use a number of antioxidants as substrates which has the advantage that a combination effect can be achieved of 1 and 2. Furthermore, it is important that no reactive species that can give rise to further deteriorative reactions are formed during this reaction. This occurs when applying other proposed oxygen-scavenging enzyme systems: glucose oxidase[2] or lipoxygenase[3] where hydrogen peroxide is formed and in the case of laccase[4,5] where reactive radical cations or quinones can be formed. Hydrogen peroxide can be effectively removed using catalase but oxygen is a reactive product in this reaction which makes the glucose oxidase/catalase system less efficient.



quercetin

2-(3',4'-dihydroxybenzoyloxy) -4,6-dihydroxybenzoic acid

Figure 1. **Reaction mechanism of quercetinase with the substrates quercetin and oxygen.**

Dioxygenases consume oxygen via a different mechanism, that is, by incorporation of oxygen atoms into their substrates, which has the advantage that no reactive species are formed. Quercetinase from *Aspergillus japonicus* is an extracellular copper containing dioxygenase that is able to incorporate oxygen to certain flavonoids, such as quercetin and kaempferol, which leads to decolourisation[6,7]. Quercetinase is a dimer, having a molecular weight of 110 kDa, containing two identical monomers of 55 kDa and containing 1 Cu molecule per monomer. Catechinase, also from *Aspergillus japonicus*, uses flavonoids, such as catechin as substrate to remove oxygen. Catechinase is a monomeric Fe-containing enzyme having a molecular weight of 58 kDa (1 Fe/molecule enzyme). Since dioxygenases are very substrate-specific and thus it rarely happens that the food product contains the substrate naturally, substrates have to be added. These substrates are often antioxidants; for example catechinase and quercetinase can use catechin and quercetin as substrates, respectively. This is shown in the reaction mechanism of the reaction of quercetinase with quercetin and oxygen, given in Figure 1 and the reaction of catechinase with catechin in Figure 2.

The reaction scheme shows that 1 molecule of quercetin or catechin is needed for the consumption of 1 molecule of

* To whom correspondence should be addressed. E-mail: liesbeth.bouwens@unilever.com.

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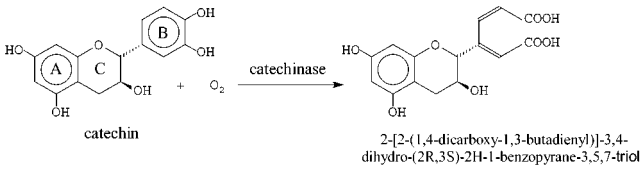

Figure 2. **Reaction mechanism of catechinase with catechin and oxygen.**

oxygen. Only in the reaction with quercetinase, one molecule of CO is formed for each molecule of oxygen that is consumed. The fact that during the quercetinase reaction oxygen is replaced by carbon monoxide might be a important disadvantage for the use of quercetinase in food products.

Quercetinase and catechinase are both extracellular fungal dioxygenases which are obtained by performing a 100-L fermentation of *Aspergillus japonicus* using respectively quercetin and catechin (10 g/L) as inducer.[8] Overexpression of quercetinase and catechinase in *Aspergillus awamori* delivered good quantities of enzyme (at least 0.5 g/L; van der Helm et al, unpublished data). They are unique because in general dioxygenases, such as catechol dioxygenases and protocatechuate dioxygenase, are part of complex intracellular multienzyme systems, which are often membrane-bound and require external cofactors. In contrast, quercetinase and catechinase do not depend on an external cofactor. The crystal structure of quercetinase (quercetin 2,3-dioxygenase)[9] and the characterisation of catalytic states of the enzyme by EPR[10] became available only recently. The structure and kinetics of catechinase have not yet been investigated.

The dioxygenase/antioxidant combination might be a good method to use for deoxygenation of food and nonfood products which are sensitive to oxidation, particularly in emulsions such as mayonnaise and spreads. The aim of this work is to scope the use of the combination of oxygen-scavenging dioxygenases and antioxidants for the prevention of autoxidation of food products, focused on oil-in-water emulsions. Potential usage of dioxygenases for enhanced stability and shelf life (but also the antioxidant value) will be discussed. The emphasis is on the use of dioxygenase/antioxidant combinations for the reduction in off-flavour formation which will be evaluated analytically, by measuring oxygen levels and analyzing the volatiles which are representative for off-flavour and, more close to the consumer, by surveying taste-perception by means of a taste panel of experts. The effect of deoxygenation of products on the antioxidant properties will be discussed using antioxidant activity assays.

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Materials

YSI biological oxygen monitor, oxygen (model 5300), oxygen electrodes, and oxygen permeable membranes were used. YSI Bath Assembly, from Yellow Springs Incorporated Co., U.S.A connected with pen recorder and water bath 25 °C was used.

HPLC model HP1090 with diode array detector from Hewlett-Packard, U.S.A. was used. RP-HPLC column: ODS Hypersil C18 column: 3 μm (4.6 mm × 100 mm) and precolumn ODS Hypersil C18 column: 5 μm (4.6 mm × 10 mm).

Gas chromatograph model GC-17A with Static-Headspace and FID detector was used from Shimadzu. A capillary CP-Sil 5CB column (length 25 m, i.d. 0.25 mm, o.d. 0.39 mm, film thickness 1.2 μm) from Chrompack was used.

Enzymes. Quercetinase from *Aspergillus japonicus* 2.1 mg/mL and catechinase from *Aspergillus japonicus* 0.84 mg/mL, both overexpressed in *Aspergillus awamori*.[8] Both enzymes were identified, produced, and isolated (>90% pure, stored in 30 mM MES buffer pH 6 at −80 °C) at Unilever R&D Vlaardingen, The Netherlands.[9]

Substrates/Antioxidants. Quercetin dihydrate, (+)-catechin hydrate, and kaempferol were from Fluka Belgium, and instant green tea (IGT) was from Unilever R&D Colworth, UK.

Oil. Safflour/linseed oil (SA/LN 85/15) was obtained from Chempro, The Netherlands, and extra virgin olive oil (first press) was obtained from Puget, Vitrolles, France.

Methods

Oxygen Consumption Assay. Oxygen levels were determined using an biological oxygen monitor, oxygen electrodes, and oxygen-permeable membranes calibrated with air-saturated water at 25°. Oxygen uptake (removal) by the addition of enzymes (5.6 mg/L catechinase and 0.14 mg/L quercetinase) was measured in time, and initial oxygen uptake activity was calculated. For catechinase, catechin was added in an amount of 1 mM (0.3 g/L). For quercetinase, quercetin was added as substrate in an amount of 1 mM (0.3 g/L) The specific activity of quercetinase is expressed in units (U) defined as μmol min^{-1} mg^{-1} of oxygen consumed by quercetinase. The activity of quercetinase for the indicated batch was: 293 μmol min^{-1} mg^{-1} oxygen consumed and for catechinase: 6.4 μmol min^{-1} mg^{-1} oxygen consumed.

Analysis of Reaction Components by HPLC. Samples were analysed on reverse phase HPLC. An ODS Hypersil column (C18) 3 μm 4.6 mm × 100 mm was used for analytical determination of the reaction components (e.g., quercetin and catechin). A linear gradient of solvent A (2% acetic acid/2% acetonitrile/96% Milli Q water pH 2.8) and solvent B (100% acetonitrile) was used under the following conditions: $t = 0$ min 100% solvent A, $t = 30$ min 40% solvent B, flow: 1 mL/min. Sample preparation was as follows: samples were 1:1 diluted in a block solution (60% CH_3CN/10%HAc/30% MilliQ) to stop the reaction. Prior to injection the samples were centrifuged for 10 min at 14 000 rpm using an Eppendorf centrifuge. Sample (20 μL) was injected on the column using an autosampler. Calibration of the components was performed using external standard

calibration with catechin, detection at 280 nm, and quercetin at 360 nm. Reference samples with catechin and quercetin were checked to be clearly resolved. The retention times (t_R) of the elution of catechin, quercetin and their reaction products were: t_R (2-[2-(1,4-dicarboxy-1,3-butadienyl)]-3,4-dihydro-2H-1-benzopyrane-3,5,7-triol) = 5 min, t_R (catechin) = 6 min, t_R (2-(3′,4′-dihydroxybenzoyloxy)-4,6-dihydroxybenzoic acid) = 12.5 min and t_R (quercetin) = 20 min.

Preparation of Emulsions. Emulsions were made with 40% oil (SA/LN 85/15), 59% w/w buffer (0.15 M adipine acid pH 5) and 1% w/w/ Tween 60 using an Ultra-Turrax. Two series of experiments were carried out for off-flavour analysis by static headspace gas chromatography and the following samples were:

(1) The effects of quercetinase (17 mg/L) and the substrate quercetin (6 g//L or 20 mM) were tested, flushed, and filled with pure oxygen in the headspace. Samples were made of 17 mL of headspace and 3 mL of emulsion. The samples were capped and incubated for 24 h at 40 °C and then further incubated at 60 °C for 14 days.

(2) Effects of quercetinase (0.014 mg//L)/quercetinase (1.5mM) and catechinase (0.14 mg//L)/catechin (1.5mM) were tested in air-filled headspace. Samples were made of 10 mL of headspace and 10 mL of emulsion. The samples were capped and incubated for 24 h at 40 °C and then further incubated at 60 °C for 4 weeks.

Static-Headspace Gas Chromatography (GC) Analysis. The lipid oxidation of samples was followed by static-headspace GC analysis of the following volatiles: acetaldehyde, propenal, propanal, pentane, 2-tert-butenal, 1-pentene-3-one, 1-pentene-3-ol, pentanal, pentenal, and hexanal. The column used was a capillary CP-Sil 5 CB column. The following settings for the autosampler and for the chromatograph were used so that the volatiles were well resolved. The GC Cycle time for one sample is 25 min including the cool step to 0 °C.

Temperature program: Maximum temperature: 399 °C. Initial temperature 0 °C. Inititial time 4.00 min. Rate 40 °C/min. Final temperature 40 °C. Final time 0.00 min. Rate A 5.0 °C/min. Final temperature A 60 °C. Final time A 0.00 min. Rate B 40 °C/min. Final temperature B 200 °C. Final time B 3.00 min. Total program time 15.5 min.

Pressure program: Initial value:115 kPa. Initial time: 4.00 min. Rate: 10 kPa/min. Final pressure: 125 kPa. Final time: 0.00 min. Rate A: 1.5 kPa/min. Final pressure A:131 kPa. Final time A: 0.00 min, Rate B: 10.3 kPa/min. Final pressure B: 167 kPa. Final time B: 3.00 min.

Detector FID: Range: 0. Polarity: 2. Current: 0. H_2 Flow: 50 mL/min. Air flow: 500 mL/min. Make up gas flow: 30 mL/min.

Mayonnaise Preparation. Citric mayonnaise was made according to a standard protocol for the preparation of mayonnaise. The following ingredients were used: 80% vegetable oil, egg yolk, water, vinegar, sugar, glucose, syrup, mustard, nutritious acid E-270, spices, pigment E-160a, aroma components. Quercetin (2 g) was added to 6 kg of mayonnaise, and as the last ingredient 6 mg of quercetinase was added just before the mayonnaise passed through the colloid mill. Glass jars were filled completely, almost without a headspace, and immediately closed. The pH of the citric mayonnaise was 3.4.

Table 1. **Oxygen uptake activity of dioxygenases in 25 mM acetate buffer pH 6.0 and in 60% o/w emulsion (olive oil/acetate buffer) using 2 % (w/w) Tween as emulsifier**

	[enzyme] (mg/L)	oxygen uptake activity[a] [μmol O_2/min·mg] 0% oil	60% oil	substrate
quercetinase	0.14	293 ± 22	266 ± 34	1 mM quercetin
		351 ± 2	284 ± 42	1 mM kaempferol
catechinase	5.6	6.99 ± 0.07	6.74 ± 0.46	0.3% (w/v) IGT[b]
		6.40 ± 0.61	6.84 ± 1.41	1 mM catechin

[a] $[oxygen]_{initial}$ (mM) for 0% oil samples was 0.29 mM and for 60% oil samples was 0.80 mM. [b] IGT = Instant green tea.

Antioxidant Activity Assay. Antioxidant activity was measured using the standard Trolox equivalent antioxidant capacity (TEAC) assay and the ferric reducing ability of plasma (FRAP) method as described by Miller, N. J. et al[11] and Benz, I. F. F. et al.,[12] respectively. Samples were made of 0.1 mM quercetin or catechin with and without enzyme addition in concentrations of 0.1 mg/L quercetinase or 10 mg/L catechinase, respectively, in 20% methanol and 80% demineralised water, pH 6. Samples were incubated for 60 min at room temperature. In the samples with enzyme, the product formation was checked to be 100% using HPLC analysis.

Results

Activity of Dioxygenases. The activity of the two dioxygenases, quercetinase and catechinase, with and without the presence of oil is shown in Table 1.

As shown from Table 1 the dioxygenases are both active in oil in water emulsions, even up to 75% oil (not shown). The presence of oil did not inhibit the enzyme significantly. The specific uptake activity of quercetinase per mg enzyme is 40−50 times higher compared to that of catechinase which makes quercetinase more preferable for applications (less enzyme dosage, low cost price). Quercetinase is most active towards kaempferol, which is in agreement with the results of Oka.[13]. They found the reactivity of the substrate is greatly influenced by the distribution of the hydroxyl substituents (kaempferol 4′ OH at B-ring where quercetin 3′- and 4′-OH at B-ring, see Figure 1).

The optimal activity of both dioxygenases is in the pH range 5−6.5 (data not shown). Their activity at low pH (the relevant pH for spreads and dressings) is given in Table 2.

Although the activity of both dioxygenases decreased at more acidic conditions, quercetin was most stable and showed to be still 92% active at pH 3.5. Thus, oxygen consumption by quercetinase can be applied in products with

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Table 2. **Activity of quercetinase and catechinase at low pH[a]**

pHst	activity quercetinase [μmol O_2/min·mg]	activity catechinase [μmol O_2/min·mg]	% activity catechinase	% activity quercetinase
3.5	298 ± 3	4.8	32	92
4	319 ± 19	8.6	57	98
5	324 ± 7	15.2	100	100

[a] Activity at pH 5 was taken as 100 % value.

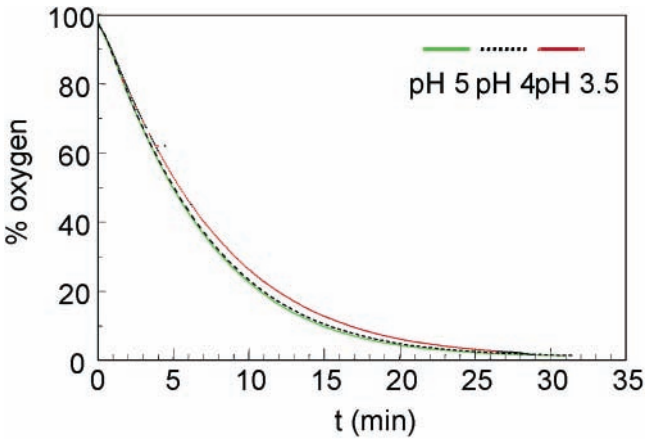

Figure 3. **Oxygen consumption in time by quercetinase (0.14 mg/L) in 12.5% w/v oil-in-water emulsions with 1 mM quercetin, 2% w/v Tween at pH 3.5, 4, and 5.**

an acidic pH: for instance mayonnaise (pH 3.8), spreads (pH between 3.8 and 4.5). As shown, catechinase is also active at low pH but less than quercetinase. Compared with that of queretinase, the specific activity of catechinase was already lower at pH 5 and decreased even more at pH 3.5 which makes this enzyme less preferable.

The oxygen concentration could be reduced to zero by the addition of the enzymes as shown in the example in Figure 3 where oxygen concentration against time is given of oil-in-water emulsions in the presence of quercetinase and quercetin. This demonstrates the potential usage of enzymes for a deoxygenation of products on a molecular level, whereas others (e.g., deoxygenation via packaging or filters) often reduces oxygen slowly to a certain amount.

The dioxygenases are active at room temperature. Their initial activity was most optimal at 40 °C, and they are still very active at 60 °C.

Stability. Both dioxygenases are very stable; at room temperature their activity remained about 100% for 50 days. At higher temperatures (till 60 °C) quercetinase was still 100% active after 24 h incubation at 60 °C, and catechinase kept 85% of its activity. Both enzymes were unstable at 80 °C, within 10 min activity decreased to zero. In presence of 0.5% NaCl their activity remained about 95 %. In presence of 60% oil their activity was 100%. When 0.5% NaCl was added to a 60/40 o/w emulsion, at the local NaCl concentration of 1.2%, their activity remained 80%. The excellent stability towards salt is a clear benefit for the use of dioxygenases in applications as spreads and dressings, especially since other enzymes (as laccases) are generally very unstable under these circumstances.

Table 3. **Antioxidant activity of the substrates quercetin and catechin of quercetinase and catechinase, respectively, and their reaction products**

samples	TEAC (mM)	FRAP (mM)
quercetin	0.44	0.31
quercetin + quercetinase	0.44	0.39
catechin	0.38	0.32
catechin + catechinase	0.35	0.24

Antioxidant Activity. The presence of antioxidants is a key factor in off-flavour formation, and therefor the antioxidant activity of the used antioxidants was measured in the presence and absence of the dioxygenases. Antioxidant activity is difficult to quantify since it can be directed towards the prevention of various radical reactions. We have chosen to use two assays: the Trolox equivalent antioxidant capacity (TEAC) assay and the ferric reducing ability of plasma (FRAP) assay; the data of the antioxidant activity are given in Table 3.

The antioxidant activity of quercetin is similar to the antioxidant value of 2-(3′,4′-dihydroxybenzoyloxy)-4,6-dihydroxybenzoic acid which is the product of the quercetinase reaction with quercetin. This means that there is no loss of the antioxidant value during the reaction!

The antioxidant value of catechin showed a small decrease when oxygen was incorporated in catechin by the enzyme catechinase, but the product contained antioxidant value. This shows that both the antioxidant activity of the substrate as its oxidation product can contribute to prevent off-flavour formation.

The antioxidant active moiety of catechin and quercetin is the B ring described by Jovanovic et al.[14] They concluded that the ring whose radical has the lowest reduction potential is the antioxidant moiety in any flavenoid. Since the incorporation of oxygen in quercetin by quercetinase occurred at the C ring, the antioxidant moiety of the B ring remained intact, although the reduction potential of the B ring is likely to be different. In the case of catechin the decrease of antioxidant value could be due to the fact that the incorporation of oxygen by catechinase did occur at the B ring.

Off-Flavour Prevention: Oil-in-Water Emulsions. The production of off-flavour of 40/60 oil-in-water emulsions was measured in time in absence and presence of quercetin with and without quercetinase using static headspace GC analysis. The experiment was done under standard conditions for optimal off-flavour generation using a large amount (85% v/v) of headspace filled with pure oxygen and a high incubation temperature of 60 °C. To scavenge all the oxygen present it was needed to add a high antioxidant concentration (20 mM). The amount of the volatiles of the samples after 15 days is given in Table 4.

As shown in the reference sample high levels of off-flavour were produced. The combination of quercetin and

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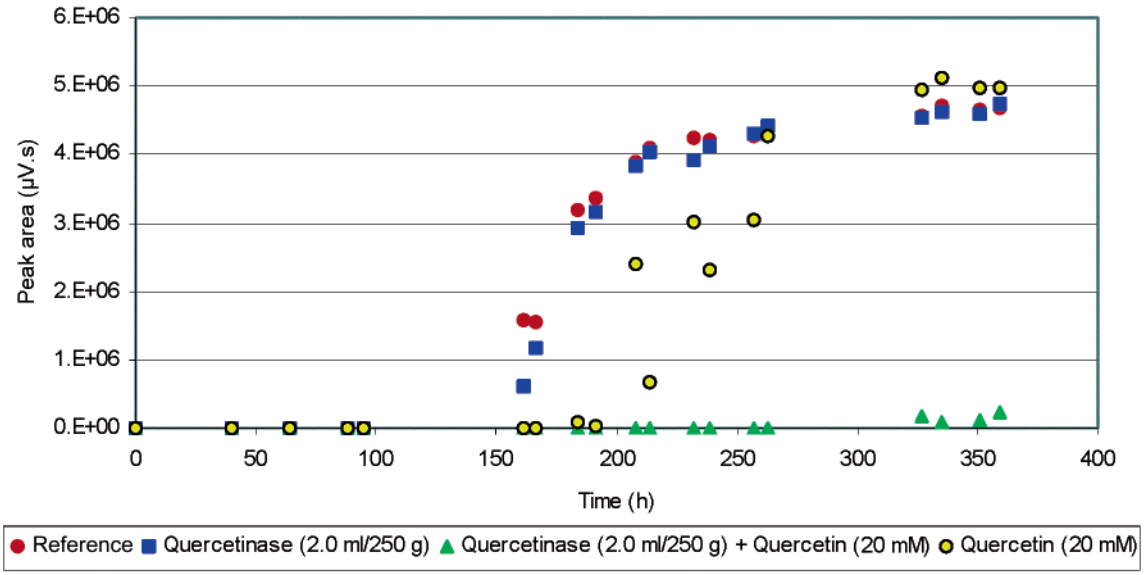

Figure 4. **Pentane production, representative for most flavour components in oil: 40/60 o/w emulsion at 60 °C (40% SA/LN, 1% Tween 60, 59% pH 5 adipic acid (0.15 M) buffer solution).**

Table 4. **Flavour production (in ppm) after 15 days incubation of 40% o/w emulsions filled with pure oxygen**

	acetaldehyde	propenal	pentane	2-*tert*-butenal	1-pentene-3-ol	pentanal	pentenal	hexanal
reference	67.5	1.1	243.4	7.9	17.9	337.7	37.3	1627.1
quercetin + quercetinase	4.4	0.4	12.5	1.1	4.2	6.7	8.2	84.2
quercetin	73.3	4.5	258.9	12.9	28.4	100.4	68.3	923.9

Table 5. **Flavour production (in ppm) after 4 weeks incubation of 40% o/w emulsions filled with pure oxygen**

samples	acetaldehyde	propenal	pentane	pentanal	hexanal
reference	3.0	9.8	35.3	2.3	17.5
quercetin/ quercetinase	1.6	8.3	33.2	0.9	8.2
quercetin	3.6	9.6	29.9	1.5	12.3
catechin/ catechinase	0.7	6.5	43.1	1.4	13.3
catechin	1.7	8.5	32.2	1.3	10.3

quercetinase addition not only reduced the formation of the volatiles, it also prevented the formation of almost all volatiles! The addition of quercetin alone reduced the volatiles for some extent. However after 2 weeks incubation at 60 °C the addition of quercetin led to enhanced off-flavour production for most volatiles and reduced only the production of two volatiles. The formation of pentane, which is a representative off-flavour component, is shown as function of time in Figure 4.

A second experiment was carried out under more realistic storage conditions for food products using an air-filled headspace instead of pure oxygen and lower concentrations of antioxidants and enzyme. In this experiment both quercetinase and catechinase were tested on their ability to prevent off-flavour formation. In this experiment the formation of 10 volatiles was analysed, but only five volatiles were produced at reasonable levels. The amount of these volatiles in the samples after 4 weeks is given in Table 5. As shown the combination of either quercetinase (0.014 mg/L)/quercetin (1.5 mM) or catechinase (0.14 mg/L)/catechin (1.5 mM) prevented the formation of three of the five volatiles, while in the reference sample high levels of these volatiles were produced.

Other volatiles were produced in very low concentrations.

Off-Flavour Prevention in Mayonnaise. For the following taste experiment four citric mayonnaise samples were prepared, filled in glass jars almost without a headspace:

sample 1	no additions
sample 2	with 1 mM quercetin and 1 mg/L quercetinase
sample 3	with 1 mM quercetin
sample 4	with 1 mM EDTA

Sample 4 is the standard mayonnaise with the addition of EDTA as a metal scavenger and can be considered as positive control. The mayonnaise was stored at 5 °C, at room temperature (20 °C), and at 37 °C.

Quercetin levels were analysed by RP-HPLC analysis and showed that in sample 2 quercetin was oxidised for 90%. This indicates that quercetinase is very active in the mayonnaise product. The mayonnaise samples were judged for rancidity every 2 weeks during a total period of 16 weeks by three experienced persons. The taste results of the products stored at 5, 20, and 37 °C are presented in Table 6 of which the data of the 5 °C storage trial is also presented in Figure 5.

As shown the mayonnaise without additives was not stable as shown by the rapid decrease of the taste scores. The addition of only quercetin addition had a slight positive effect. The addition of the combination quercetin/quercetinase enhanced the stability. As shown in Figure 5, the taste of

Table 6. **Results of the taste of mayonnaise by a taste panel: high numbers mean a better taste and less off-flavour (score 8 = good, score 5 = off)[a]**

	1. no additives			2. quercetinase + quercetin			3. quercetin			4. EDTA		
storage time (weeks)	5 °C	20 °C	37 °C	5 °C	20 °C	37 °C	5 °C	20 °C	37 °C	5 °C	20 °C	37 °C
2	8	8	6	7.5	7	6	7.5	6.5	5.5	8	8	8
4	7.5	6.5	6.5	8	8	6.5	8	6.5	5	8	8	7
6	6.5	6.5	5	8	6.5	5.5	7.5	8	5	8	8	7
8	5.5	6.5	n.d.	8	8	5	8	8	5	8	7.5	6
10	6	5.5		7	7	n.d.	8	6	n.d.	8	7	5
12	6	5		7	6		6	5		8	7	n.d.
16	5	n.d.		8	7		5	5		8	6.5	

[a] n.d. not determined because sample was too rancid.

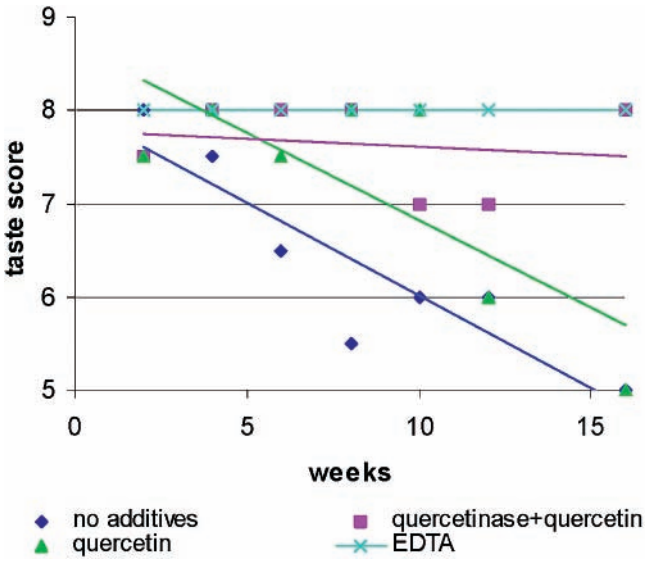

Figure 5. **Stability of mayonnaise samples, stored at 5 °C.**

the mayonnaise with quercetin and quercetinase was observed to be good during the whole 16 weeks storage at 5 °C. The best prevention of off-taste was observed when EDTA was added.

On the basis of these results, especially at 5 and 20 °C, it is concluded that the combination quercetin and quercetinase gives good protection against oxidation, while quercetin alone does not give a sufficient result (results were comparable with the reference, sample 1). EDTA addition provided the highest off flavour prevention; however, EDTA is not suited as general additive for mayonnaise (not accepted in all countries).

Conclusions

The use of dioxygenases, quercetinase and catechinase, as oxygen-scavenging enzymes to prevent off flavour in oil-in-water emulsions was found to be highly efficient. The enzymes achieve their antioxidant effect, in part, by reducing the oxygen concentration in the sample to zero, so that no autooxidation can take place. In combination with the antioxidant activity of the substrate and its oxidation product, this has resulted in a decreased formation of off-flavours in a model system. The combination of quercetinase and quercetin resulted in very effective off-flavour reduction of 80−97% as determined by GC analysis of 7 out of 10 volatiles, while in the references the off-flavour caused volatile increase.

As a practical example the effect of quercetinase/quercetin on off-flavour formation in mayonnaise was tested. On the basis of the taste results it is concluded that quercetinase/quercetin gave good protection against oxidation, while quercetin alone did not.

Furthermore, the enzymes are highly stable in oil-in-water emulsions, active at low dosage levels (0.01−0.1 mg/L for quercetinase, 0.1−1 mg/L for catechinase), stable against salt (0.5% NaCl), and still active at relatively low pH, that is, pH 3.5−5 which is relevant for application in spreads and dressings.

Discussion

Despite the fact that the combination dioxygenases/antioxidant as oxygen-scavenging method to prevent off-flavour in oil-in-water emulsions was found to be highly efficient, the use of the dioxygenases tested have major bottlenecks: they are both not food-grade, and therefore safety clearance is required. However, even if the enzymes are proven to be safe for consumption, the fact that they are produced via recombinant DNA technology might hamper their application in food products due to public opinion. Quercetinase is preferable over catechinase because it can be added at very low levels. However, the fact that carbon monoxide is formed in the quercetinase reaction might be a disadvantage for the use of this enzyme in food which has to be investigated further in terms of concentrations, and so forth. Catechinase would be a better alternative. Another bottleneck is the limitation on the levels of antioxidant which may be added to a product. However, levels of 1 mM (0.3 g/L) quercetin or catechin are allowed and in combination with dioxygenases (0.01−0.1 mg/L quercetinase or 0.1−1 mg/L catechinase) found to be effective for prevention of off-flavour generation. Therefore we recommend using these conditions in products where deoxygenation on a molecular level is desired. Furthermore, they can be used in vitamin and antioxidant-containing products to prevent them from being oxidised as, for instance, in tomato paste containing vitamin C, or in nonfood products containing antioxidants, such as retinol in skin cream.

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