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(54) Title: METHOD TO PREVENT OXIDATION OF COMPONENTS IN OIL, AND METHOD TO REDUCE THE USE OF ETHOXYQUIN TO PREVENT OXIDATION OF COMPONENTS IN OIL

(57) Abstract: A method is described for the prevention of oxidation of components in an oil, where berries or fruit are crushed to a pulp of berries and that the pulp of berries is then pressed for the separation into two phases, a juice phase and a press residue, and that the press residue is added to an oil exposed to oxidation. Also described is a method to reduce the use of ethoxyquin in marine oils or animal feed, and also a press residue with a high content of anthocyanidines and a method for the production thereof.



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TITLE

5 Method to prevent oxidation of components in oil, and
method to reduce the use of ethoxyquin to prevent
oxidation of components in oil.

FIELD OF THE INVENTION

10

The present invention relates to a method to prevent
oxidation of components in an oil, where a press residue
from berries or fruits is added to the oil. The invention
also relates to a method to reduce the amount of ethoxy-
15 quin which is added to an oil to prevent oxidation of
components in the oil where a press residue from berries
or fruit is added to the oil. Furthermore, the invention
comprises a composition to prevent oxidation of oil.

20 BACKGROUND AND PRIOR ART

In the production of diluting juices and pure juice from
fruits and berries the juice fraction is separated from
the press residual fraction. In general, mills are used to
25 crush the berries and it is also normal to increase the
yield of juice fraction by heating gently. After a
thorough mixing and crushing of the berries, and after a
certain incubation time, the mass is pressed and the two
phases are separated. The juice fraction is used for the
30 manufacture of, for example, diluting juices and pure
juice, while the press residue is normally considered to
be a waste product.

It is known that fruits and berries contain large amounts
35 of antioxidants, but it has been shown that only a small
proportion of these antioxidants can be found as active

antioxidants in the finished diluting juice or pure juice. Either antioxidants are damaged by the processing steps that are necessary to give a high juice yield, or the antioxidants can be found in the solid fractions that are
5 part of the press residue.

Thus it is an aim of the present invention to produce a fraction from berries and fruit that contains active antioxidants. In particular, it is an aim of the present
10 invention to maintain any possible anti-oxidative activities in the press residue, as it must today be considered to be a waste product.

It is also an aim of the present invention that the press
15 residue can be processed to a dry material, preferably in powder form, for example by freeze drying, and that the press residue in its dry and preferably storage stable form maintains the activity of the antioxidants which originally are in the berry and fruit material.

20 It has surprisingly been found that different preparation of the press residue provides different proportions of antioxidants in the final product. This is measured by the amount of antocyanidines present after processing. Thus it
25 is an aim of the present invention to improve the processing methods after isolation of the press residue, and it is of particular interest to use the press residue that gives the best activity with respect to prevention of oxidation of the oxidation vulnerable components of an
30 oil, preferably marine oil.

Furthermore, it has surprisingly been shown that the relationship between the amount of press residue and anti-oxidant effect is not linearly proportional. Normally one
35 would expect that if one increases the addition of a press residue then the anti-oxidising activity also will

increase correspondingly. However, tests have shown that if the amount exceeds a given size it will start an oxidation, i.e. that the anti-oxidising effect is reduced and countered. The press residue is a heterogeneous material, and in this material several anti-oxidants cooperate, and it is assumed, without being bound by theory, that some of these components will have an oxidising effect in certain amounts. It is therefore important to determine the optimal concentration range for the effect of the press residue in relation to the material (oil) which shall be treated so that one adds sufficient amount until one achieves a good anti-oxidant effect, but at the same time one is outside the area, i.e. below the concentration, which gives a pro-oxidative effect.

Today artificial anti-oxidants are widely used to prevent oxidation of oils. In particular, marine oils which contain significant amounts of unsaturated fatty acids (incorporated in complex lipids) are very susceptible to oxidation. To reduce this oxidation the anti-oxidant ethoxyquin is usually added which is a quinoline-based anti-oxidant which is used for the preservation of food products as a special addition to prevent fats becoming rancid. There is currently some speculation regarding possible adverse health effects of ethoxyquin, and the FDA (U.S. Food and Drug Administration) has requested feed producers to use smaller amounts of ethoxyquin.

Thus ethoxyquin is a problem in the food industry today, and there is therefore a wish to find other anti-oxidants that can reduce the level of, or make completely redundant, ethoxyquin in feed products.

Inventors of the present invention have conducted tests that show one can reduce the amount of ethoxyquin if one

adds a press residue from berries or fruit. There is reason to believe that the different anti-oxidants in the press residue will affect different components of the oil, and that this reduces the need for the addition of ethoxyquin. By a sufficient addition of press residue the amount of unwanted ethoxyquin can thus be reduced significantly because of a synergistic interaction between ethoxyquin and the various components from the press residue.

SUMMARY

A first aspect of the present invention relates to a method to prevent oxidation of components in an oil, where berries or fruit are crushed to a pulp of berries, and that the pulp of berries is thereafter pressed for separation into two phases, a juice phase and a press residue, after which the press residue is frozen or dried, characterised in that the press residue is thereafter added to said oil where the amount of press residue which is added to the oil is determined so that the amount is sufficient for the press residue to provide an anti-oxidising effect on one or more components of said oil and that the amount is below a level that brings about an oxidising effect on said oil.

The pulp of berries is preferably incubated during and after crushing at a temperature of 20-40 °C for a period of 2-24 hours.

In a preferred embodiment enzymes are added during incubation, preferably pectin degrading enzymes.

It is preferred that the press residue is frozen, preferably at a temperature of -20 °C, or that the press residue is dried to a dry material, preferably in powder

form. In a preferred embodiment the press residue is freeze dried under vacuum, preferably under an inert atmosphere, preferably nitrogen or carbon dioxide.

5 In a preferred embodiment the press residue has a content of anti-oxidants in the range 400 to 1200 ppm, more preferably in the range 600 to 1200 ppm, more correspondingly in the range 800 to 1200 ppm, more preferably in the range 900 to 1100 ppm, more preferably
10 in the range about 1000 ppm, related to phenols.

In a preferred embodiment said press residues prevent release (hydrolysis) of fatty acids from complex lipids, such as phospholipids and mono-, di- and triglycerides in
15 said oil.

In a preferred embodiment said press residues prevent the oxidation of fatty acids, where said fatty acids are mono- or polyunsaturated.

20 One preferred embodiment comprises prevention of oxidation of EPA or DHA.

In one embodiment said oil is of marine origin.
25

In preferred embodiments the press residue is from blackcurrant, blueberry or apple.

In a preferred embodiment the amount of press residue that
30 is sufficient so that the press residue has an anti-oxidising effect on one or more components in said oil is determined and at the same time is below a level that brings about an oxidising effect on said oil, in that the inhibited oxidation of different amounts of press residue
35 in a given amount of marine oil under similar conditions (time, temperature) is determined to find the optimal

amount of press residue for a given oil.

Preferably the optimal range is measured using one
parameter or method chosen from AUC (to be specified),
5 TOTOX and resistance index.

In a second aspect the present invention relates to a
method to reduce the amount of ethoxyquin which is added
to an oil to prevent oxidation of components in the oil,
10 characterised in that a press residue from berries or
fruit is added to said oil.

In a preferred embodiment the quantity of press residue is
in one amount sufficient for the press residue to inhibit
15 the oxidation of one or more components in said oil, and
that the quantity is below a level that brings about an
oxidising effect on said oil.

In a preferred embodiment the amount of press residue in
20 said oil is in the range 400 to 1200 ppm, more preferably
in the range 600 to 1200 ppm, more correspondingly in the
range 800 to 1200 ppm, more preferably in the range 900 to
1100 ppm, more preferably in the area around 1000 ppm,
related to phenols and that the amount of ethoxyquin is in
25 the range 0-400 ppm ethoxyquin.

In a preferred embodiment the amount of press residue in
said oil is in the range 400 to 1200 ppm, more preferably
in the range 600 to 1200 ppm, more correspondingly in the
30 range 800 to 1200 ppm, more preferably in the range 900 to
1100 ppm, more preferably in the range about 1000 ppm,
related to phenols and that the amount of ethoxyquin is in
the range 0-400 ppm ethoxyquin, more preferably 0 to 300
ppm, and more preferably 0 to 200 ppm.

In a preferred embodiment the addition of press residue reduces the need for addition of ethoxyquin by 30%, more preferably 40% and more preferably 50%.

- 5 In a preferred embodiment said residues prevent the release (hydrolysis) of fatty acids from complex lipids, such as phospholipids and mono-, di- and triglycerides in said oil.
- 10 In a preferred embodiment the press residue prevents oxidation of fatty acids, preferably mono- or polyunsaturated fatty acids, preferably polyunsaturated.

In a preferred embodiment the press residue prevents
15 oxidation of EPA or DHA.

In a preferred embodiment said oil is of marine origin.

In preferred embodiments said press residue is from
20 blackcurrant, blueberry or apple.

A third aspect of the present invention relates to a composition to prevent oxidation of an oil, characterised in that it contains;

- 25 i) a press residue of berries or fruit and
ii) ethoxyquin.

In a preferred embodiment the amount of press residue is sufficient for the press residue to have an anti-oxidising
30 effect on one or more components in said oil and that the amount is below a level that brings about an oxidising effect on said oil.

In a preferred embodiment the relative amount of press
35 residue to ethoxyquin is approx. 1:1, more preferably approx. 2:1, more preferably 3:1, more preferably 4:1 ,

more preferably 5:1, more preferably more than 5:1 such that for example, 10:1 or 15:1 or 20:1 or ratios in between.

5 In a further aspect the invention relates to a press residue from berries or fruit, where said berries or fruit are crushed to a pulp of berries and that the pulp of berries is then pressed for the separation into two phases, a juice phase and a press residue, characterised
10 in that the press residue is thereafter freeze-dried or vacuum dried and has a content of antocyanidines as follows;

Delphinidine, more than 1500, more preferably more
15 than 1550, and most preferably more than 1650
Cyanidine, more than 1800, more preferably more than 1900, and most preferably more than 1950
Petunidine, more than 850, and more preferably more than 900
20 Peonidine, more than 150, and more preferably more than 190
Malvidine, more than 1900, and more preferably more than 1950;
where all quantities are expressed as mg per 100 g of
25 press residue.

In a further aspect the invention relates to a method for production of a press residue in accordance with claim 36, characterised in that berries or fruit are crushed to a
30 pulp of berries and that the pulp of berries is then pressed for the separation into two phases, a juice phase and a press residue, characterised in that the press residue is thereafter freeze dried or vacuum dried.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be described in more detail with reference to the accompanying figures, where:

5

Figure 1 shows OxyConc analyses (O_2 consumption by incubation for 40 min at 40 °C) of 100 grams of press cake (Baltic herring) to which freeze-dried blueberry powder (fBB) (from example 1) has been added corresponding to 1000, 2000 or 4000 ppm phenols. Control samples are "without anti-oxidant" (-AO) and Ethoxyquin (FEQ) (100 ppm), respectively.

10

Figure 2 shows OxyConc analyses (O_2 consumption during incubation for 20 + 20 hours at 40 °C) of 100 g herring press cake (Baltic herring) with added blueberry press residue corresponding to 1000, 2000 or 4000 ppm phenols. Control samples are without anti-oxidant (-OA) and FEQ500 (100 ppm), respectively.

15

20

Figure 3 shows the parallels from the respective fractions of the OxyConc analyses.

25

Figure 4 shows a dose response curve for a measured amounts of freeze dried blueberry press residue (fBB) containing brisling oil and incubated for 166 hours at 40 °C in an air flow.

30

Figure 5 shows the amount of free fatty acids in oil samples to which measured amounts of freeze dried blueberry press residue (fBB) is added and incubated for 166 hours at 40 °C in an air flow.

35

Figure 6 shows the reduction in fatty acid groups in oil samples to which measured amounts of freeze-dried blueberry press residue (fBB) is added and incubated for 166 hours at 40 °C in an air flow.

5

Figure 7 shows the development of PV and AV, respectively, at the start and after 30 days.

10

Figure 8 shows the consumption of oxygen for 50 hours at different concentrations of fBB in a press cake from the herring.

15

Figure 9 shows the induction time of blackcurrant press residue at 65 degrees where 35 grams of powder is used as the raw material.

20

Figure 10 shows the effect of press residue from apples corresponding to 1000 ppm phenols on the degradation of EPA and DHA through 101 days of storage at 40 °C.

Figure 11 shows the induction time of an apple press residue at 65 degrees where 35 grams of powder is used as raw material.

25 Experimental part

Example 1

Manufacture of press residues

30 Ripe blueberries are sorted free of any mildew, rot and leaves. Thereafter they are crushed in a mill and the pulp of berries is heated to 20-40 °C. Pectin degrading enzymes are added to the pulp of berries, stirring constantly. After a reaction time of 2-24 hours, depending on the
35 temperature of the mass, the pulp is pressed. Rotary presses of the type Bucker are used. The juice yield is 75

to 88% dependant on berry type and degree of ripening. The amount of press residue varies from 12-25%.

Manufacture of the press residue in powder form

5

The press residue is produced as described above. After pressing in the rotary press, the press residue is frozen down to about -20 degrees C before further processing.

10 The frozen press residue is thawed slowly, and then freeze dried. Nitrogen gas was added when the vacuum was removed. After drying, the products were finely ground into a powder using a Retch-mill. The water content was determined in the respective powders.

15

The powder was thereafter kept under a blanket of nitrogen before testing. The samples were stored in plastic boxes with about 125 grams per box and stored in a freezer at -20 degrees C.

20

Analysis of the content of antocyanidine in different preparations of blueberry

25 The content of antocyanidines in different preparations was determined using conventional methods, and the different values are given in Table 1.

Table 1

30 The content of antocyanidines from different preparations of blueberry (mg/100mg)

Sample	Delphinidin	Cyanidin	Petunidin	Pelargonidin	Peonidin	Malvidin	Total
Blueberry	121	154	68	0	17	168	527
Freeze Dried	1682	1959	911	0	199	1961	6710
Vacuum Dried	1570	1842	870	12.9	196	1904	6396
Raw material?				0			
Mill Dried	1444	1725	812	25	183	1785	5972

Example 2

- 5 Effect of blueberry press residue in a press cake from
Baltic Sea herring

OxyCons analysis

- 10 It is measured that 1 gram of blueberry press residue
(fBB) contains 11.17 mg of phenols and 39.2% dry matter.
fBB was added in given amounts to a press cake (PC) made
from Baltic herring at Karmsund Fiskemel AS after
treatment in a screw press (portions of 600 grams). The
15 amounts measured correspond to three different levels of
phenols from fBB; 1000 ppm, 2000 ppm and 4000 ppm (mg/kg
PC). After mixing and homogenisation, the samples were
dried in a fluid-bed dryer (hot air blowing through at 105
°C) to a water content of 8%. The dried samples were
20 transferred to flasks for OxyCons analysis (measuring O₂
consumption during incubation at 40 °C for 40 hours). The
OxyCons analysis was conducted in five samples (two
parallels per sample), i.e. that besides the samples
containing fBB two control samples were run where

ethoxyquin (100 ppm FEQ500) containing 50% ethoxyquin (synthetic antioxidant) and 50% formic acid is added to one, and also a sample without the addition of anti-oxidant. Table 2, shown below, shows the total weight and water content of dried and ground press residues, produced as described above.

Table 2

	Press residue - before drying		Press residue - after drying	
Blueberry	2200	41	907	1.68

10

The dry material content (DM) in freeze-dried blueberry press residue (fBB) was 98.32%, while the analysis results showed that the DM content in the press residue of blueberries before freeze-drying was 40.5%.

15

Calculations showed that the phenol content in 1 gram of fBB was 27.12 mg. The DM content of the press cake made from Baltic herring was 33.6%.

20

The amount of fBB which was added per 600 grams PC was 7.2, 14.8 and 29.6 grams, respectively, which corresponds to 1000, 2000 and 4000 ppm phenols of dry substance in the PC, respectively.

25

After drying in a fluid-bed dryer the OxyCons analysis was conducted in the respective samples. Atmospheric O₂ content in the respective flasks was logged every 15 minute for 40 hours.

30

Figure 1 shows the results from the OxyConc analysis. The results of the parallels are acceptable (see below). The

results show that the sample that did not contain anti-oxidants (-AO) had the highest consumption of O₂. We also see that the second control sample that contained FEQ500 gave the lowest O₂ consumption.

5

However, it was surprisingly found that the sample to which an amount of fBB corresponding to 1000 ppm phenols was added had approximately the same O₂ level as the sample to which FEQ500 was added after 40 hours incubation. This corresponds with the results of "non-freeze dried" blueberry (produced as described above). These results are shown in Figure 2. The fact that the largest anti-oxidant effect is found in the sample that contains the least amount of press residue, i.e. equivalent to 1000 ppm phenols, is very surprising.

10
15

It is thus possible to determine the lowest amount of press residue that gives a high anti-oxidative activity with a relatively low consumption of press residue. The results show the same ratios for both freeze-dried and non freeze-dried press residue, i.e. the water activity of the press residue is not decisive for the anti-oxidative function.

20

25 Conclusion

The experimental results show that the press residue from blueberries contains active components that protect marine lipids against oxidation. This activity is maintained despite the press residue being freeze dried and finely ground, and that the sample is exposed to heat.

30

The anti-oxidant activity improved as the amount of press residue used was reduced, i.e. as one went from the press residue corresponding to 2000 ppm and 4000 ppm phenols, respectively, to 1000 ppm phenols.

35

Furthermore, it can be seen in Figures 1 and 2 that the curves with low content of press residue, i.e. the sample corresponding to 1000 ppm phenols, shows the anti-oxidative effect to last longer, and this is seen as a flattening out of the curve. This is particularly evident in Figure 2 where one sees that the curve for fBB corresponding to 1000 ppm phenols actually cuts the curve for FEW500 at an incubation time of just less than 40 hours. The same effect can be seen in Figure 1, but here the point of intersection is later.

Example 3 - fBB has an anti-oxidative effect on marine oil (brisling oil)

Manufacture of fish press cake and fish oil

Press cake free of anti-oxidant and oil was produced from fresh brislings. Brisling is among the fattest industrial fish species and has a high content of polyunsaturated fatty acids such as EPA, DHA and DPA. Unsaturated fatty acids are easily oxidised and to preserve the oil's nutritional quality it must be protected against oxidation by the addition of anti-oxidants.

80 kg of fresh brisling of nutritional quality was bought from Norway Pelagic Bergen on 08.09.2009 and stored overnight in a refrigerator at 4 °C. The brisling was boiled for 15 min in water (1:1) in a total of 3 servings. The boiling water was reused. After the coarse sieving, the fish mass was fed into a twin screw extrusion press (Stord Bartz Type P9, 5 screw speed: 2 rpm). The sieve and press fluid were heated to 95 °C and run through a liquid strainer (Type Jesma) with an aperture of 100 microns before the oil was separated by a continuous self-cleaning separator (Westfalia type). The oil was stored in a refrigerator at 4 °C prior to the test (14.09.2009) in a

nitrogen atmosphere.

The press cake of fresh brislings was weighed, mixed in, and vacuum packed in portions of approximately 600 grams.

5 The press cake portions were stored in a refrigerator at 4 °C.

Freeze-dried blueberry press residue (fBB) was produced as described in example 1. The powder was kept cool at 4 °C
10 prior to the test. The solids content was measured at the start of the experiment to check if the powder had absorbed water during the storage at 4 °C.

Butylated hydroxytoluene (BHT) is a synthetic anti-oxidant
15 permitted for use in food. BHT is added to all fish oils. The threshold for maximum concentration of BHT in oils is 150 ppm on its own. BHT is registered with the CAS number 0000128370. In this study, BHT from Sigma-Aldrich (product No. 000000000000047168) was used.

20

Pure standards of natural anti-oxidants isolated from fruits and berries, cyanidine 3-O- β -glucopyranoside from Polyphenols AS Hanaveien, 4-6, 4327 Sandnes, Norway
25 (Product No. 1201-3) were used.

25

Accelerated oxidation

Accelerated oxidation of marine oil was simulated in an experimental setup comprising: a water bath, an air pump
30 and air flow meters. The method was based on the protocol for Active Oxygen Method (AOCS Official Method Cd 12-57).

Test glasses with an oil sample were placed in a water bath set at 40 °C. The air speed was adjusted so that the
35 through flow of air was similar in all tests, about 3 ml/sec $\pm$ 10%. The air speed was slightly higher than

recommended in the AOCS method. The air speed was controlled at regular intervals. All the tests were run simultaneously, a total of 10 glasses. 25 ml oil was taken out at each sampling. One sample representing all the samples was taken at the start to characterise the oil quality prior to the test.

Chemical analysis

AV - anisidine value (anidisine number) is a standard procedure, accredited according to international standard ISO 17025 and registered under the internal number A09 (edition 1.6). The method determines the amount of secondary products formed from free fatty acids in oil as a result of lipid oxidation. The reaction between aldehydes (especially 2-alkenales) and p-anisidine.

PV - peroxide value (peroxide number) is a standard procedure, accredited according to international standard ISO 17025 and registered under the internal number A 10 (edition 1.3). The method determines the amount of primary oxidation products formed in the oil as a result of oxidation.

$$\text{TOTOX} = \text{AV} + 2 * \text{PV}$$

FFA - free fatty acids, a standard procedure, accredited according to international standard ISO 17025 and registered under the internal number A07 (edition 1.6). The method determines the amount of acid groups formed in the shape of secondary oxidation products.

Fatty acid composition - a standard procedure, accredited according to international standard ISO 17025 and registered under the internal number A68 (edition 1.4). The result is used to get an overview of the relative

amounts of each class of fatty acid and can be related to the nutritional value of the oil product.

Antioxidant effect of fBB in marine oil

5

The test was started on 14.9.2009 and finished on 21.9.2009. The test for accelerated oxidation in marine oils described above was used.

- 10 Measured amounts of fBB were added to 200 ml of brisling oil so that the concentration of phenols from fBB in the oil was about 100, 250, 500, 750 and 1000 ppm.

- 15 The oil samples were placed in a water bath and incubated at 40 °C for 166 hours (approximately 7 days). Samples of 25 ml of oil were removed after 22, 70 and 166 hours. Lipid oxidation in brisling oil to which was added measured amounts of fBB was compared to brisling oil without anti-oxidant or added 100 ppm BHT, or added 100
20 ppm cyanidine 3-glucoside (section 2.1.4) and with the oil sample taken prior to the incubation (0 hours).

Results

- 25 The following quality parameters were measured in the raw material (fresh brisling): total volatile nitrogen - 11.5 mg N/100 g, tri-methylamine-N-0 mg/100 g; tri-methylaminoxide-N - mg N/100 g, total dry matter - 29.9% and total fat (ethyl acetate extraction) - 12.2%. The
30 results confirm that the raw material was fresh and of nutritional quality. The yield from 80 kg brisling was 6 kg crude brisling oil and 19 kg brisling press cake. The brisling oil had a relatively low degree of oxidation TOTOX = 31. In this experiment anti-oxidants could not
35 have been added during the production because the effect of these should be examined in an oil free of anti-

oxidant. The sum of polyunsaturated fatty acids was 26.3 g per 100 g oil, and 25 fatty acid profiles, as shown in table 5.

5 **Parameters for the oxidation were measured with different methods**

i) area under the curve (AUC)

10 Fresh brisling oil had 0.3% free fatty acids, low levels of primary (9.5 m.eq. peroxide/kg oil) and secondary (12.0 units) lipid oxidation products which is reflected in a relatively low oxidation coefficient, TOTOX (= AV + 2xPV) of 31 units.

15 The results show that brisling oil without anti-oxidant was quickly oxidised. The TOTOX kinetics during the 166 hour incubation period can be described by a third degree polynomial function, $y = -0.001x^3 + 0.26x^2 - 5.30x + 31$ and a corresponding correlation coefficient of $R^2 = 1$. The
20 TOTOX kinetics in the remaining oil samples are described by third degree polynomials. It is common to compare regression curves with the help of their characteristic values (for example, slopes). In cases where the curves were described with different types of equations or
25 nonlinear functions the basis for comparison is more complicated. In such cases, the parameter, the area under the curve (AUC), is a way to compare results. The extent of oxidation can be interpreted visually with a value for the amount of oxidation products formed during the
30 specified time and calculated as the area under the curve (AUC) for TOTOX.

This parameter is central to the pharmacokinetics where it is used for a characterisation of the kinetics of
35 different foreign substances (medicines) in the body. AUC is used to characterise the organism's ability to

metabolise the material and thus form a basis to compare different materials. In this study, the AUC is calculated with the help of a trapezoidal model. For this, the total AUC is first divided into trapezoids with a base limited by time intervals in the experiment and heights determined by TOTOX at time 1 and time 0. The area of each trapezoid is calculated according to the formula given below. Finally, the individual AUCs are added together. Larger AUC means more oxidation products in the sample and the highest degree of oxidation.

Table 3 shows the AUC calculated for all samples. The lowest AUC was calculated for the oil stabilised with 111 ppm BHT. This was half of the AUC for the oil without anti-oxidant or stabilised with 533 ppm phenols in the form of fBB or 107 ppm of cyanidine 3-glucoside. A trend for the inversely proportional correlation between the amount of fBB (phenols) and AUC is seen in the concentration range 533 to 1066 ppm phenols. The lowest AUC for oils with added fBB was calculated for 1066 ppm phenols. Simultaneous stabilisation of the oil with 108, 268 and 804 ppm fBB phenols results in almost similar AUCs, i.e. gives almost the same protection and better than 107 ppm cyanidine 3-glucoside alone or without the anti-oxidant.

Table 3

AUC calculated for TOTOX curves for the tested oils

Antioxidant	AUC
BHT	914
3.8% fBB	1076
2.8% fBB	1480
0.4% fBB	1517

1.0% fBB	1548
Cyanidine 3-glucoside	1680
0	1683
1.9% fBB	1857

These findings confirm that the correlation between the amount of added fBB and AUC as a measure of the anti-oxidant effect is not of a linear character. A possible explanation for this phenomenon may be that the anti-oxidants that are present in the fBB material have a two-sided character, i.e. they function as both pro-and anti-oxidants. Therefore, the results obtained show that it is important that the concentration of added anti-oxidants, i.e. the number of ppm anti-oxidants in the fBB material is optimised. The amount must be above a certain level to achieve sufficient activity, but at the same time the amount must also be below a certain level to prevent a pro-oxidative effect.

15

ii) resistance index (index of resistance to rancidity, RI) is a parameter which is used to characterise the potential of the samples to resist oxidation. RI describes how long it took before the sample was oxidised to a selected PV value.

20

Table 4a

Resistance Index (RI), i.e. the number of hours before the oil was oxidised to PV = 100 milli equivalents.

5

AOX (ppm)	Antioxidant	RI (hours)
0	0	40
108	0.4% fBB	41
107	Cyanidine-3-glucoside	43
107	0.4% fBB	49
533	1.9% fBB	49
268	1.0% fBB	70
804	2.8% fBB	70
1066	3.8% fBB	84
111	BHT	90
109	BHT	98

The addition of fBB has, to a significant extent, extended the resistance of brisling oil to oxidation from 33 hours (without fBB) to 55 hours (corresponding to 1066 ppm phenols as fBB). The correlation between resistance index and the amount of added fBB was not linear as the oil with 533 ppm of phenols in the form of fBB had a lower RI than oil with 108 ppm. The highest resistance to oxidation was measured in the sample with an addition of 1066 ppm.

15

Table 4b

Resistance Index (RI), i.e. number of hours before the oil
was oxidised to TOTOX = 100

5

AOX (ppm)	Antioxidant	RI (hours)
0	0	33
107	0.4% fBB	33
107	Cyanidine-3-glucoside	33
533	1.9% fBB	34
108	0.4% fBB	36
268	1.0% fBB	43
804	2.8% fBB	44
1066	3.8% fBB	55
111	BHT	74
109	BHT	75

iii) Dose response curves for measured amounts of fBB
added to marine oil

- 10 The results show that the anti-oxidant effect of fBB is
dependant on the amount of fBB and that this correlation
is not linearly proportional. The dose response curve in
figure 4 is based on TOTOX values after 166 hours. The
curve is described by a third degree polynomial ($R^2 = 0.8$).
- 15 The curve shows that for the given configuration, it is
fBB equivalent to 500 ppm phenols that provides the least
protection. The oil sample was most oxidised (the highest
TOTOX) and highest concentration of free fatty acids (data
not shown).

20

Free fatty acids (FFA)

The level of FFA is higher in oxidised samples than in
fresh and stable samples. The level of FFA in brisling oil

was 0.3% at the start. Figure 5 shows that the level of FFA in the oil without anti-oxidant has increased at least 6 times in the course of 166 hours. Two oil samples containing 100 ppm cyanidine 3-glucoside and 533 ppm phenols had higher levels of FFA than oil without anti-oxidant. This suggests that these amounts of anti-oxidants have provided the poorest protection against oxidation. The oils to which 107, 268, 804 and 1066 ppm phenols (fBB) and 100 ppm BHT were added had levels of FFA lower than the oil without any anti-oxidant. This indicates that the above amounts of fBB were sufficient to give anti-oxidant protection for the brisling oil. The observed anti-oxidant effect was not linearly proportional to the amount of fBB in the oil (which is also indicated above).

Fatty acid composition

Unsaturated fatty acids such as omega-6 and especially omega-3 account for a range of health-promoting effects from marine oils and fish. These fatty acids are also the most susceptible to oxidation. This can be confirmed by the results described below. The effect of the added anti-oxidant to protect the oil against oxidation can be found by following the concentration of the essential fatty acids in the oil.

Table 5 shows the fatty acid composition of samples before and after incubation at 40 °C for 166 hours. The total sum of all the fatty acids was 88.5 g/100 g oil at the start, of which 23.4% was saturated fatty acids, 23.4% was omega-3, and 2.2% was omega-6 fatty acids. The lipid oxidation in oil without added anti-oxidant has resulted in moderate decreases in concentrations of saturated and monoene fatty acids of 1 and 6%, respectively. In the same sample the concentrations of omega-6 and omega-3 have decreased by 32 and 68%, respectively. The original concentrations of

omega-6 or omega-3 fatty acids (PUFA) were not preserved during the experiment in some of the samples. The results show that the omega-3 fatty acids were dramatically broken down in the oil without anti-oxidant: about 1/3 of the original amount was left after 166 hours. Approximately 60% of the original amount of omega-3 was retained in the oil containing 107 and 804 ppm phenols as fBB and about 66% of the original amount was retained in the oil containing 1066 ppm phenols as fBB.

Table 5

5 Fatty acid composition of brisling oil (weight/weight percent) with different anti-oxidants added and a control sample without anti-oxidant before and after incubation for 166 hours at 40 °C in a through flow of air.

	0 hours		166 hrs						
	Without AO	Without AOX	100 ppm Cyanid-3- glycoside	0.4% fBB n=2	1.0% fBB	1.9% fBB	2.8% fBB	3,8% fBB	100ppm BHT n=2
14:0	8.0	8.0	7.8	7.8	8.0	8.4	8.0	7.5	8.1
16:0	13.7	13.5	13.2	13.2	13.5	14.1	13.6	12.9	13.7
18:0	1.5	1.4	1.3	1.4	1.4	1.5	1.4	1.4	1.4
20:0	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.2
22:0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1
16:1 n7	3.8	3.6	3.5	3.6	3.7	3.9	3.8	3.6	3.9
18:1	9.9	9.2	8.8	9.1	9.2	9.6	9.5	9.2	9.6
20:1	8.2	7.6	7.5	7.6	7.6	7.8	7.7	7.6	7.9
22:1	16.3	15.4	15.0	15.4	15.0	15.4	15.3	15.5	15.9
24:1 n9	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.7
16:2 n4	0.4	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3
16:3 n4	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
18:2 n6	1.6	1.2	1.2	1.3	1.3	1.3	1.4	1.5	1.5
20:2 n6	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2
20:4 n6	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2
18:3 n3	1.7	0.9	1.0	1.1	1.0	1.0	1.2	1.4	1.4
18:4 n3	3.7	1.5	1.6	2.0	1.6	1.6	2.0	2.4	2.7
20:3 n3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
20:4 n3	0.8	0.3	0.3	0.4	0.3	0.3	0.4	0.5	0.5
20:5 n3	5.7	1.9	2.0	2.7	1.9	1.9	2.7	3.4	3.8
21:5 n3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
22:5 n3	0.7	0.2	0.2	0.3	0.3	0.2	0.3	0.4	0.5
22:6 n3	10.4	2.6	3.0	4.3	2.9	2.7	4.2	5.9	6.4

10 Figure 6 shows the reduction in fatty acid groups after 166 hours oxidation with respect to phenols in the oil added in the form of fBB. Approximately 60% of the original amount of omega-3 was retained in the oil with 107 and 804 ppm phenols added as fBB and about 66% of the original amount was retained in the oil with 1066 ppm

phenols added as fBB.

The same trend was observed for omega-6. The smallest decrease was about 10% in oil to which 1066 ppm phenols were added in the form of fBB (Figure 6). The concentration of omega-6 PUFA in the oil containing with about 107, 268 and 804 ppm phenols added as fBB was higher than in the oil without anti-oxidant. This shows that fBB provides brisling oil with sufficient amounts of natural anti-oxidants to protect the oil against oxidation and with this preserves the most valuable lipid components - essential fatty acids. Therefore, brisling oil to which fBB was added has a higher nutritional value than oil without added anti-oxidant. The observed effect was linear to the amount of fBB in the oil in the concentration range 533-1066 ppm phenols.

Example 4 - anti-oxidant properties of press residues from berries and fruits

Production of press residues from fruit and berries

Press residues were produced as given in example 1. The freeze drying was conducted in a vacuum plate freeze drier of the type Christ gamma 1-16. Martin Christ Gefriertrocknungsanlagen GmbH, 37507 Osterode am Harz, Germany. Frozen press residues were stored at -20 °C until processing. The freeze drying took place at an ambient temperature of +25 °C for two days and +28 °C during the last two days, a total of 4 days. The sample was ground in a Retsch mill with a 0.5 mm sieve. The freeze dried product was sealed in a plastic bag, cooled and stored at -20 °C.

Production of press cake from herring

The press cake of herring was taken directly from the press at Karmsund Fiskemel AS on 2.23.2010. The press cake

was taken out right after the press and before the addition of the greaves. The material was packed in a plastic bag, frozen at -20 °C and transported at about -9 °C. The press cake was stored at -20 °C for 5 days before
5 the test began.

At the start of the test, press cake from herring was thawed and immediately ground in a meat grinder with a 6 mm hole disc. The amount of added freeze dried press
10 residue was calculated from the dry material content in the press residue and the press cake and converted to amount of phenols. For press residues from blueberry and blackcurrant it is estimated 27.12 mg of phenols per gram of dry material. Mixtures of press cake from herring and
15 press residues from blackcurrants, blueberries and apples, respectively, were dried in a fluid-bed dryer at 105 °C to a water content of about 8%. Dried press cake with added press residues was then ground up in a coffee grinder and distributed into sealed plastic containers. The samples
20 were stored at -20 °C until chemical analysis and the Oxipres test were carried out. At the same time storage tests were started in that 50 g of sample was placed in a plastic container (not airtight) and stored at +40 °C for 30 days.

25

Example 4a - press residue from blueberries

One finds an effect of press residue from blueberries as an anti-oxidant with respect to the peroxide value (PV) and anisidine value (AV) through storage at 40 degrees for
30 30 days. Figure 7 shows the development of PV and AV, respectively, throughout the storage period. In this figure the anisidine number is most important as it will rise with the passage of time because of components that develop PV will be converted to components that develop
35 AV. This means that the oxidation has a high rate because of high peroxide value and that none of the antioxidants

tested works completely optimally, and has become overloaded because of the time and temperature. The added anti-oxidants shall remove peroxides so that the anisidine number does not rise. An addition of blueberry press residue corresponding to 800 ppm phenols provides the best protection.

Table 6 shows the breakdown of two important fatty acids through 30 days of storage at 40 degrees. There is a relatively large degradation of fatty acids through the storage period. The table shows that the herring meal with 400 and 800 ppm blueberry phenols added comes out best together with the control of ethoxyquin (EQ).

15

Table 6

Effect of anti-oxidants from fBB (blueberries) on the breakdown of fatty acids after 30 days storage at 40 °C

Breakdown of fatty acids during 30 days storage at 40 degrees		
	Percent broken down 20:5 n-3	Percent broken down 22:6 n-3
100 ppm BB-30	62	68
200 ppm BB-30	57	61
400 ppm BB-30	20	25
800 ppm BB-30	23	25
1000 ppm BB-30	24	27
100 ppm EQ-30	21	24

20

In figure 8 we see the consumption of oxygen during 50 hours. We see a clear effect of the amount of added blueberry press residues. There is very little oxygen consumption in the tests with added 800 and 1000 ppm blueberry phenols, respectively. There is a clear positive effect of the addition of blueberry phenols.

25

Example 4bEffect of press residue from blackcurrant (FBC) on
oxidation in the press cake from herring

5

Press residue from blackcurrant was produced in a way corresponding to that for blueberries, and is given hereafter as BC. Table 7 shows the effect of BC on the breakdown of fatty acids after 30 days of storage at 40 °C.

10

Table 7

Effect of anti-oxidants from BC (blackcurrant) on the degradation of fatty acids after 30 days storage at 40 °C

Breakdown of fatty acids during 30 days storage at 40 degrees		
	Percent broken down 20:5 n-3	Percent broken down 22:6 n-3
100 ppm BC-30	10	17
200 ppm BC-30	12	20
400 ppm BC-30	8	14
800 ppm BC-30	6	11
1000 ppm BC-30	10	13
100 ppm EQ-30	10	16

15

Without the addition of BC there is relatively large degradation of the two fatty acids tested. It appears that BC is very effective in preventing this degradation and the press residue from the blackcurrant is far more efficient than the corresponding press residue from the blueberries.

20

In figure 9 we see the consumption of oxygen during 100 hours. We see a clear effect of the amount of added blackcurrant phenols. There is very little oxygen consumption in all the samples and it is approximately similar. A magnification of the picture shows that the stability increases with increasing addition of

25

blackcurrant phenols. There is a clear positive effect from the addition of press residue from the blackcurrant. Blackcurrant seems to have better stabilising properties than blueberries with respect to oxidation of herring powder.

Example 4c

Press residue from apples

Freeze dried press residue from apples was produced in a way corresponding to the one used for blueberry and blackcurrant, and is expressed hereafter as "apple". The effect on the fatty acids is shown in Figure 10, and consumption of oxygen is shown in Figure 11

Example 5

Synergistic effect of ethoxyquin (EQ) and press residue from blueberries to inhibit degradation of fatty acids.

Herring meal with an added mixture of blueberries and ethoxyquin was stored for 101 days at 40 degrees, as opposed to blackcurrant and blueberry which were stored for 30 days. Figure 12 shows the effect of EQ alone and together with blueberry phenols on the breakdown of EPA and DHA during 101 days of storage at 40 °C. Table 8 shows the synergy effect of the EQ and fBB on the breakdown of fatty acids during 101 days of storage at 40 degrees Celsius

Table 8

Synergy effect of EQ and fBB on the breakdown of fatty acids during 101 days of storage at 40 degrees Celsius

5

Breakdown of fatty acids during 101 days storage at 40 degrees		
	Percent broken down 20:5 n-3	Percent broken down 22:6 n-3
100 EQ+400 fBB	6	7
400 ppm fBB	16	23
300 ppm EQ-500	2	4
200 ppm EQ-1	9	15

We see from Figure 12 and Table 8 that a mixture of 100 ppm EQ and 400 ppm fBB corresponds to between 200 and 300 ppm EQ. It appears from the present data that there is a good synergy effect between the anti-oxidants. Thus, it is possible to replace part of the EQ with blueberry press residue without compromising the anti-oxidant properties.

In figure 13 we see the consumption of oxygen during 50 hours. We see from the curves that EQ-100 and EQ 75 mixed with 400 ppm fBB has a better effect than EQ-300. This synergy between the anti-oxidants confirms the results shown in Table 5. Therefore, a mix of EQ and blueberry press residue appears to have a very good stabilising effect.

Definition of terms

Anti-oxidising effect: Ability to prevent oxidation of a material. In particular, lipids such as unsaturated fatty acids are susceptible to oxidation, and an aim of the invention is to prevent such oxidation. Unsaturated fatty acids can be in a free form, or they can be incorporated

into more complex lipids such as phospholipids or mono, di and triglycerides.

5 Pro-oxidative effect: In relation to the term "anti-oxidising effect" the term "pro-oxidative" describes an effect where the overall anti-oxidant effect is reduced and one sees a contribution of an opposite, i.e., oxidising, effect on the material.

10 The term "components" in the marine oil shall cover all components that are prone to oxidation, and preferably (but not limited to) fatty acids that may be in a free form, or they can be incorporated into more complex lipids such as phospholipids or mono-, di- and triglycerides. The
15 term also covers other components (i.e., not just fatty acids or fatty acid units) such as phospholipids, mono-, di-and triglycerides and other complex chemical compounds. In some cases, the anti-oxidising effect prevents hydrolysis, for example, that fatty acids are hydrolysed
20 from complex lipids.

CLAIMS

1. Method to prevent oxidation of components in an oil,
5 where berries or fruit are crushed to a pulp of berries
and that the pulp of berries is then pressed to be
separated into two phases, a juice phase and a press
residue, c h a r a c t e r i s e d i n that the press
residue is frozen or dried, and that the press residue is
10 thereafter added to said oil, where the amount of press
residue which is added to the oil is determined such that
the amount is sufficient for the press residue to cause an
anti-oxidising effect on one or more components of said
oil and that the amount is below a level that brings about
15 an oxidising effect on said oil.

2. Method according to claim 1,
c h a r a c t e r i s e d i n that the pulp of berries
during and after crushing is incubated at a temperature of
20 20-40 °C for a period of 2-24 hours.

3. Method according to claim 1,
c h a r a c t e r i s e d i n that enzymes are added
during the incubation, preferably pectin degrading
25 enzymes.

4. Method according to one or more of the preceding
claims, c h a r a c t e r i s e d i n that the press
residue is frozen, preferably at a temperature of
30 -20 °C.

5. Method according to one or more of the preceding
claims, c h a r a c t e r i s e d i n that the press
residue is dried to a dry material, preferably in powder
35 form.

6. Method according to claim 5,
c h a r a c t e r i s e d i n t h a t t h e p r e s s r e s i d u e i s
f r e e z e d r i e d u n d e r v a c u u m , p r e f e r a b l y i n a n i n e r t
5 a t m o s p h e r e , p r e f e r a b l y n i t r o g e n o r c a r b o n d i o x i d e .

7. Method according to one or more of the preceding
c h a r a c t e r i s e d i n t h a t t h e p r e s s
r e s i d u e h a s a c o n t e n t o f a n t i - o x i d a n t s i n t h e r a n g e 4 0 0 t o
10 1 2 0 0 p p m , m o r e p r e f e r a b l y i n t h e r a n g e 6 0 0 t o 1 2 0 0 p p m ,
m o r e c o r r e s p o n d i n g l y i n t h e r a n g e 8 0 0 t o 1 2 0 0 p p m , m o r e
p r e f e r a b l y i n t h e r a n g e 9 0 0 t o 1 1 0 0 p p m , m o r e p r e f e r a b l y
i n t h e a r e a a b o u t 1 0 0 0 p p m , r e l a t e d t o p h e n o l s .

15 8. Method according one of the preceding claims,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e s
p r e v e n t r e l e a s e (h y d r o l y s i s) o f f a t t y a c i d s f r o m c o m p l e x
l i p i d s , s u c h a s p h o s p h o l i p i d s a n d m o n o - , d i - a n d
t r i g l y c e r i d e s i n s a i d o i l .

20 9. Method according to one of the preceding claims,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e s
p r e v e n t o x i d a t i o n o f f a t t y a c i d s .

25 10. Method according to claim 9,
c h a r a c t e r i s e d i n t h a t s a i d f a t t y a c i d s a r e
m o n o - o r p o l y u n s a t u r a t e d .

11. Method according to claim 10,
30 c h a r a c t e r i s e d i n t h a t s a i d f a t t y a c i d s a r e
p o l y u n s a t u r a t e d .

12. Method according to claim 11,
c h a r a c t e r i s e d i n t h a t s a i d f a t t y a c i d s a r e
35 E P A o r D H A .

13. Method according to claim 1,
c h a r a c t e r i s e d i n t h a t s a i d o i l i s o f
marine origin.

5

14. Method according to claim 1,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e
is from blackcurrants.

10

15. Method according to claim 1,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e
is from blueberries.

15

16. Method according to claim 1,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e
is from apple.

20

17. Method according to one of the preceding claims,
c h a r a c t e r i s e d i n t h a t t h e a m o u n t o f p r e s s
residue which is sufficient for the press residue to cause
an anti-oxidising effect on one or more components of said
oil and at the same time below a level that brings about
an oxidising effect on said oil is determined in that an
inhibited oxidation of different amounts of press residue
in a given amount of marine oil under similar conditions
(time, temperature) is determined to find the optimal
amount of press residue for a given oil.

25

18. Method according to claim 17,
c h a r a c t e r i s e d i n t h a t t h e a m o u n t r a n g e f o r
optimal anti-oxidant effect is measured by a parameter or
method chosen from AUC (is described in more detail),
TOTOX, resistance index.

35

19. Method to reduce the amount of ethoxyquin which is added to an oil to prevent oxidation of components in the oil, characterised in that to said oil is added a press residue of berries or fruit.

5

20. Method according to claim 19, characterised in that the amount of press residue is in a quantity sufficient for the press residue to inhibit the oxidation of one or more components of said oil, and that the amount is below a level that brings about an oxidising effect in said oil.

10

21. Method according to claim 19, characterised in that the amount of press residue in said oil is in the range 400 to 1200 ppm, more preferably in the range 600 to 1200 ppm, more correspondingly in the range 800 to 1200 ppm, more preferably in the range 900 to 1100 ppm, more preferably in the area about 1000 ppm, related to phenols, and that the amount of ethoxyquin is in the range 0 - 400 ppm ethoxyquin.

15

20

22. Method according to claim 19, characterised in that the amount of press residue in said oil is in the range 400 to 1200 ppm, more preferably in the range 600 to 1200 ppm, more correspondingly in the range 800 to 1200 ppm, more preferably in the range 900 to 1100 ppm, more preferably in the area about 1000 ppm, related to phenols, and that the amount of ethoxyquin is in the range 0 - 400 ppm ethoxyquin, more preferably 0 to 300 ppm, and more preferably 0 to 200 ppm.

25

30

23. Method according to claim 19,
c h a r a c t e r i s e d i n t h a t t h e a d d i t i o n o f p r e s s
r e s i d u e r e d u c e s t h e n e e d f o r t h e a d d i t i o n o f e t h o x y q u i n b y
30%, m o r e p r e f e r a b l y 40% a n d m o r e p r e f e r a b l y 50%.

5

24. Method according to one of the preceding claims,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e s
p r e v e n t r e l e a s e (h y d r o l y s i s) o f f a t t y a c i d s f r o m c o m p l e x
l i p i d s , s u c h a s p h o s p h o l i p i d s a n d m o n o - , d i - a n d
10 t r i g l y c e r i d e s i n s a i d o i l .

25. Method according to one of the preceding claims,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e
p r e v e n t s o x i d a t i o n o f f a t t y a c i d s .

15

26. Method according to claim 25,
c h a r a c t e r i s e d i n t h a t s a i d f a t t y a c i d s a r e
m o n o - o r p o l y u n s a t u r a t e d .

20 27. Method according to claim 25,
c h a r a c t e r i s e d i n t h a t s a i d f a t t y a c i d s a r e
p o l y u n s a t u r a t e d .

28. Method according to claim 27,
25 c h a r a c t e r i s e d i n t h a t s a i d f a t t y a c i d s a r e
E P A o r D H A .

29. Method according to claim 1,
c h a r a c t e r i s e d i n t h a t s a i d o i l i s o f m a r i n e
30 o r i g i n .

30. Method according to claim 1,
c h a r a c t e r i s e d i n t h a t s a i d p r e s s r e s i d u e i s
f r o m b l a c k c u r r a n t .

35

31. Method according to claim 1,
c h a r a c t e r i s e d i n that said press residue is
from blueberries.

5 32. Method according to claim 1,
c h a r a c t e r i s e d i n that said press residue is
from apple.

33. Composition for preventing oxidation of an oil,
10 C h a r a c t e r i s e d i n that it contains;
i) a press residue of berries or fruit and
ii) ethoxyquin.

34. Composition according to claim 33,
15 c h a r a c t e r i s e d i n that the amount of press
residue is sufficient for the press residue to cause an
anti-oxidant effect on one or more components of said oil
and that the amount is below a level that brings about an
oxidising effect on said oil.

20

35. Composition according to claim 33,
c h a r a c t e r i s e d i n that the ratio of press
residue relative to ethoxyquin is approximately 1:1, more
preferably approx. 2:1, more preferably 3:1, more
25 preferably 4:1, more preferably 5:1, more preferably more
than 5:1 such as, for example, 10:1 or 15:1 or 20:1 or
ratios in between.

30

36. Press residue from berries or fruit, where said berries or fruit are crushed to a pulp of berries and that the pulp of berries is then pressed for the separation into two phases, a juice phase and a press residue, c h a r a c t e r i s e d i n that the press residue is then freeze dried or vacuum dried and has the following content of antocyanidines;

Delphinidine, more than 1500, more preferably more than 1550, and most preferably more than 1650

Cyanidine, more than 1800, more preferably more than 1900, and most preferably more than 1950

Petunidine, more than 850, and more preferably more than 900

Peonidine, more than 150, and more preferably more than 190

Malvidine, more than 1900, and more preferably more than 1950;

where all the quantities are expressed as mg per 100 g of press residue.

37. Method for the production of a press residue according to claim 36, c h a r a c t e r i s e d i n that the berry or fruit is crushed to a pulp of berries and that the pulp of berries is then pressed for the separation into two phases, a juice phase and a press residue, c h a r a c t e r i s e d i n that the press residue is thereafter freeze dried or vacuum dried.

Figure 1

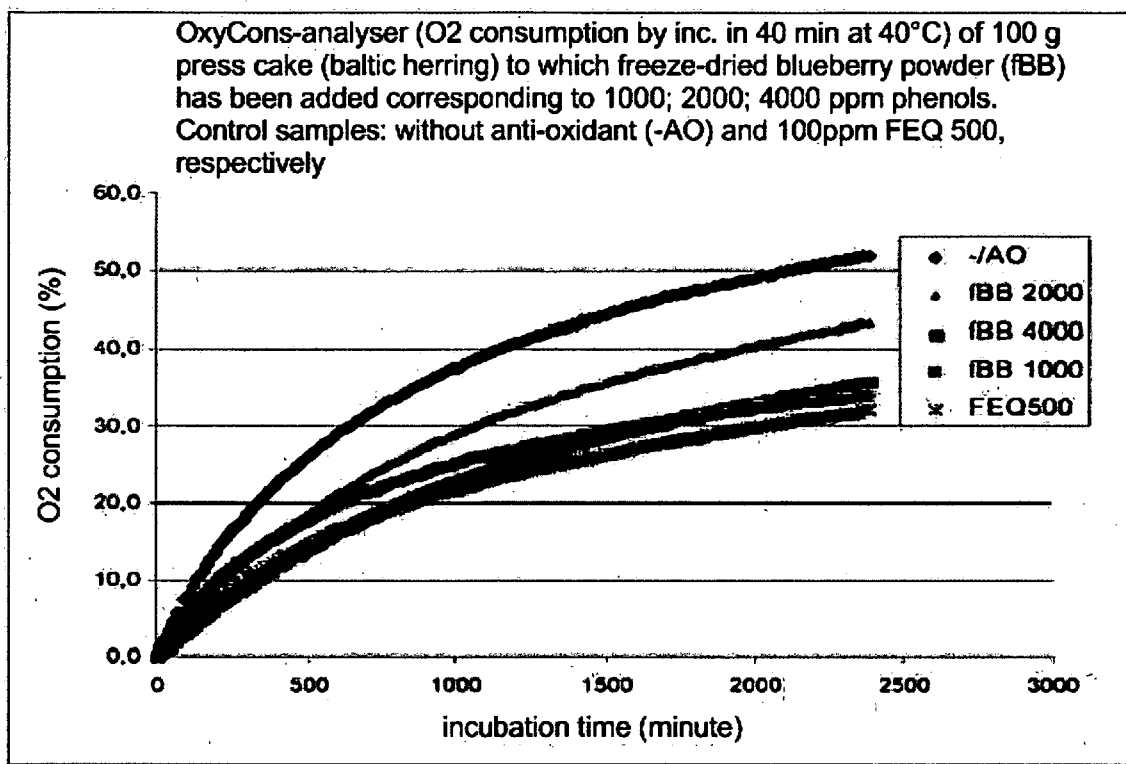


Figure 2

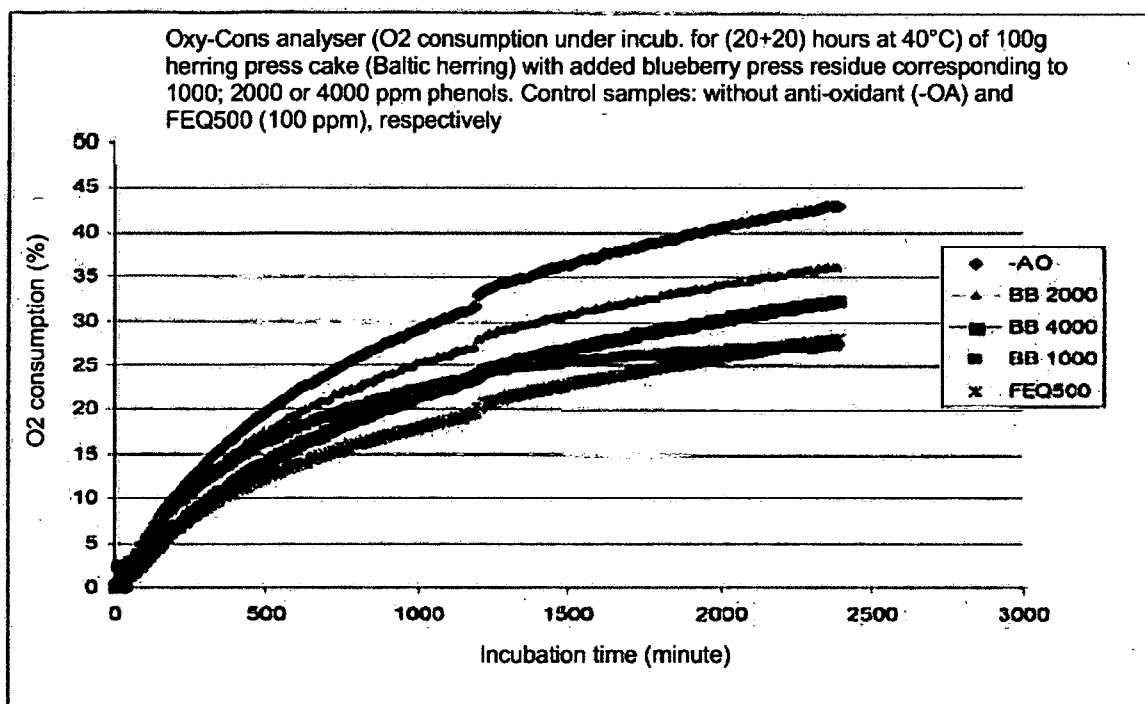


Figure 3

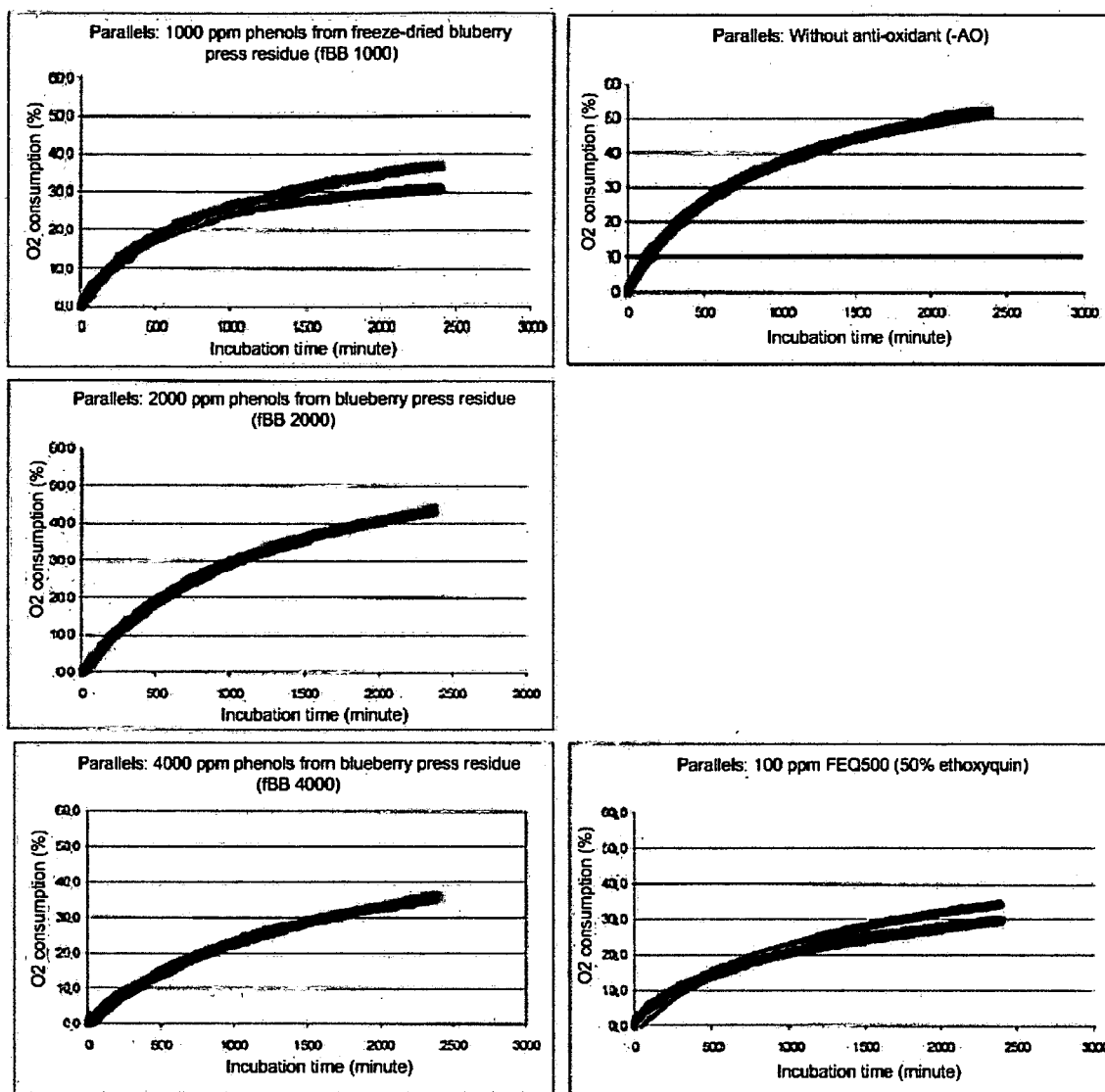


Figure 4

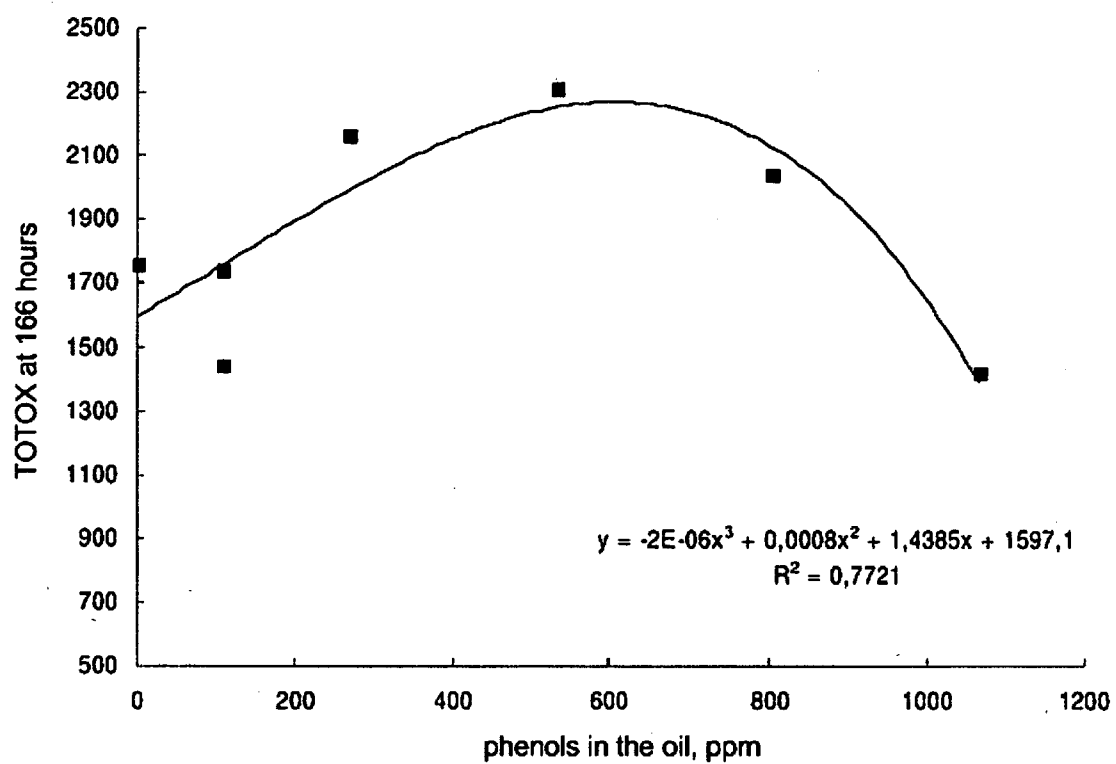


Figure 5

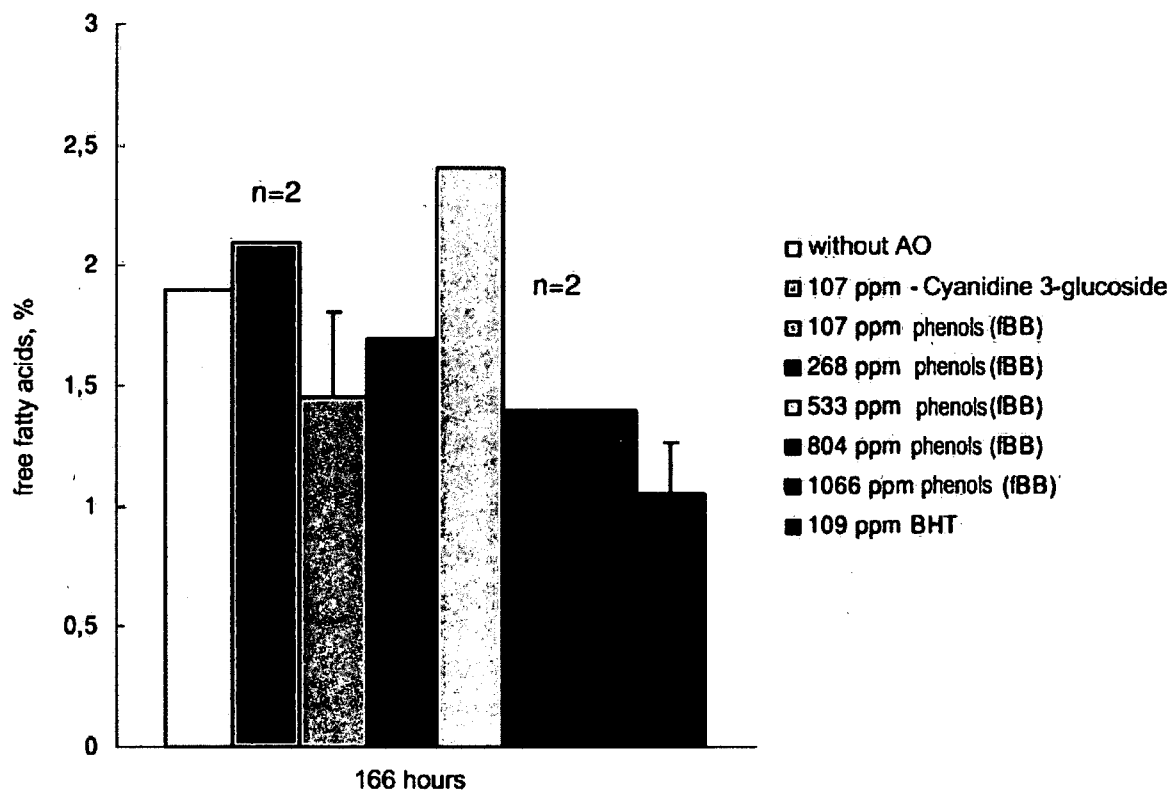


Figure 6

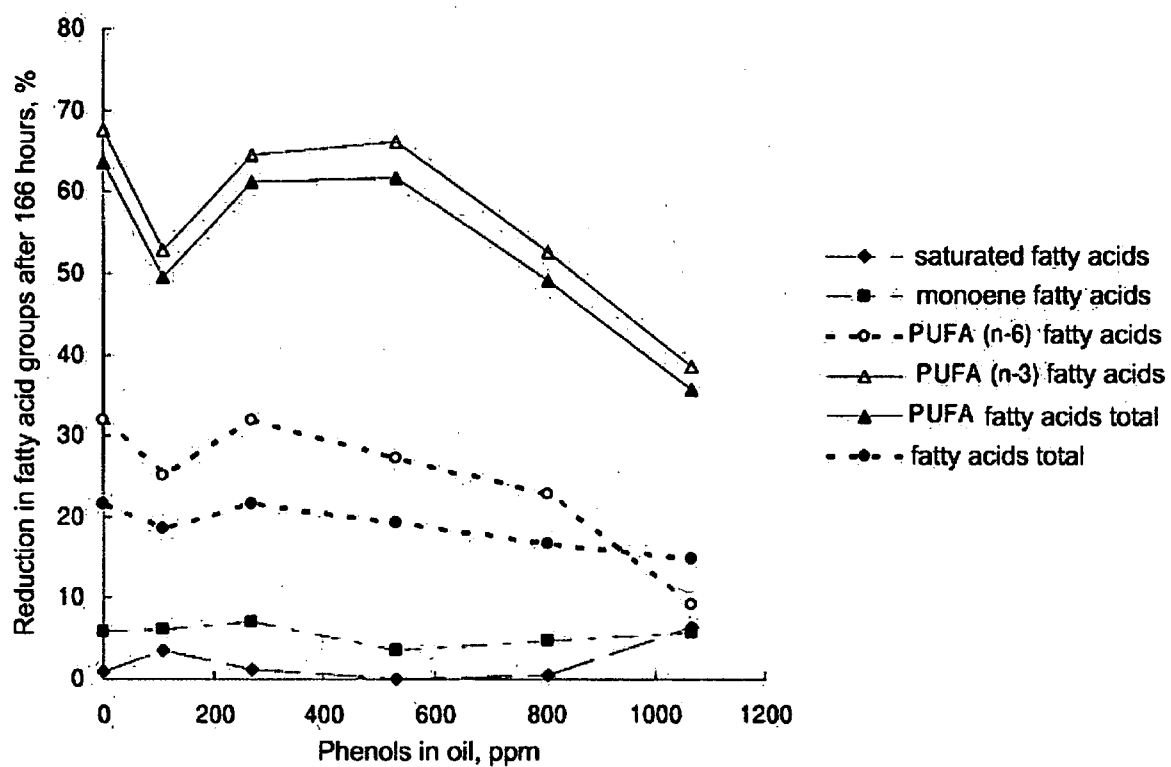


Figure 7

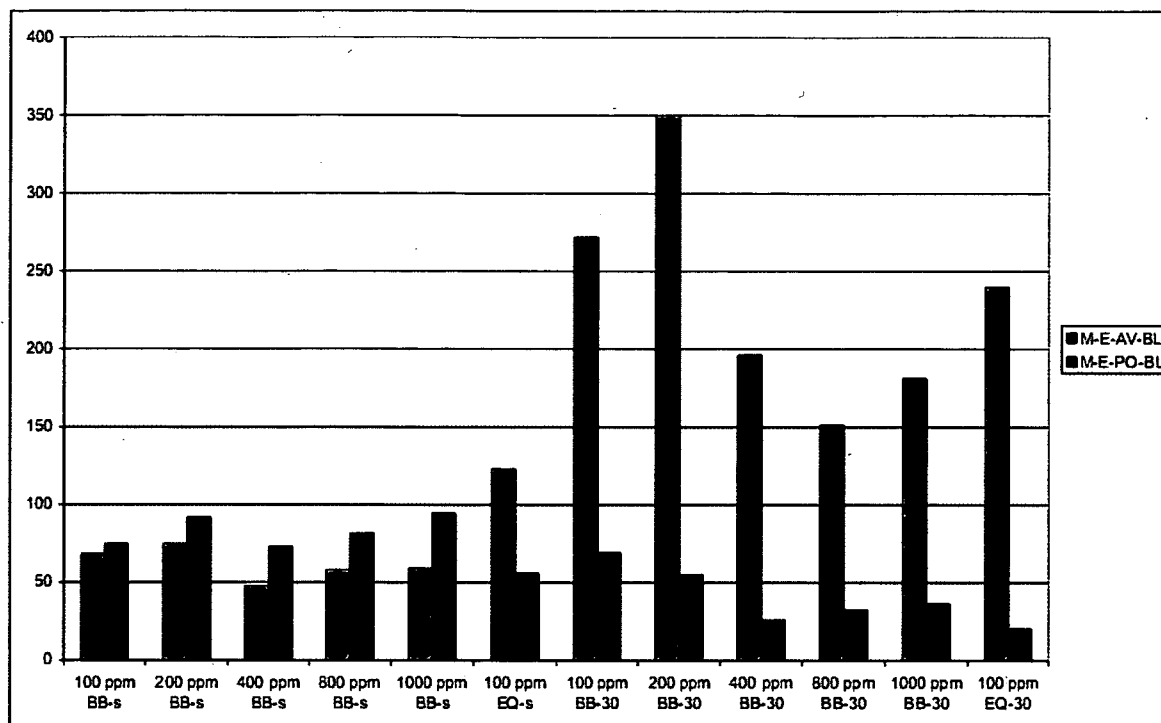


Figure 8

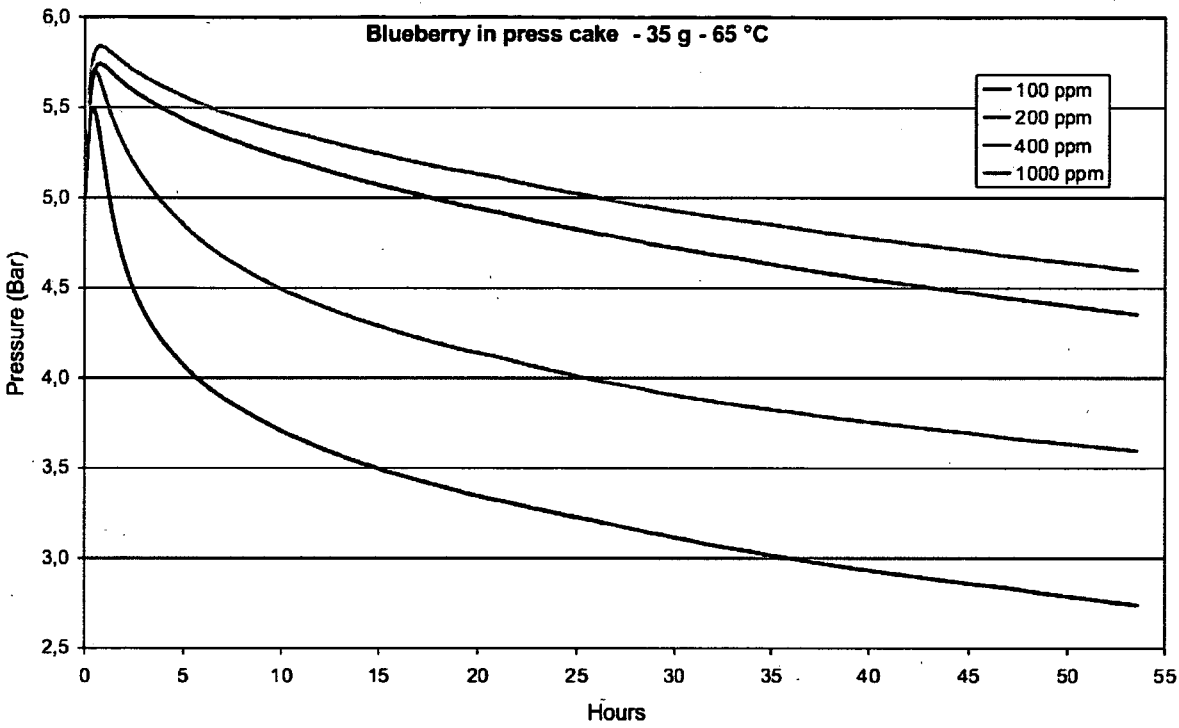


Figure 9

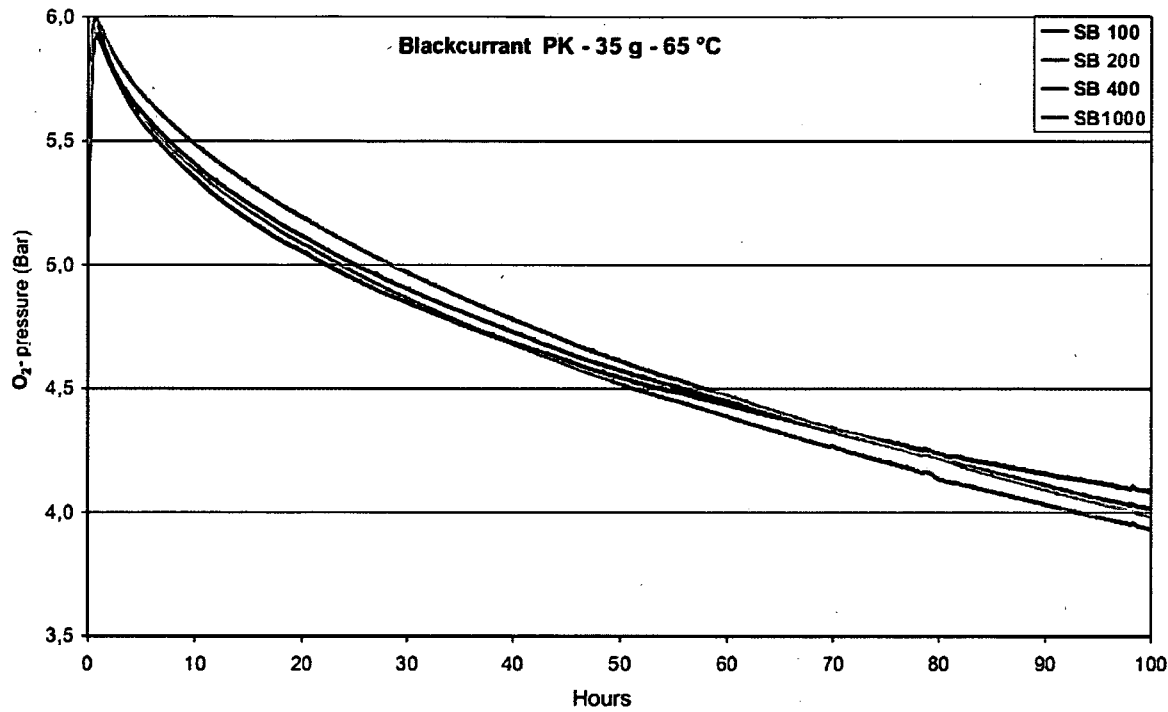


Figure 10

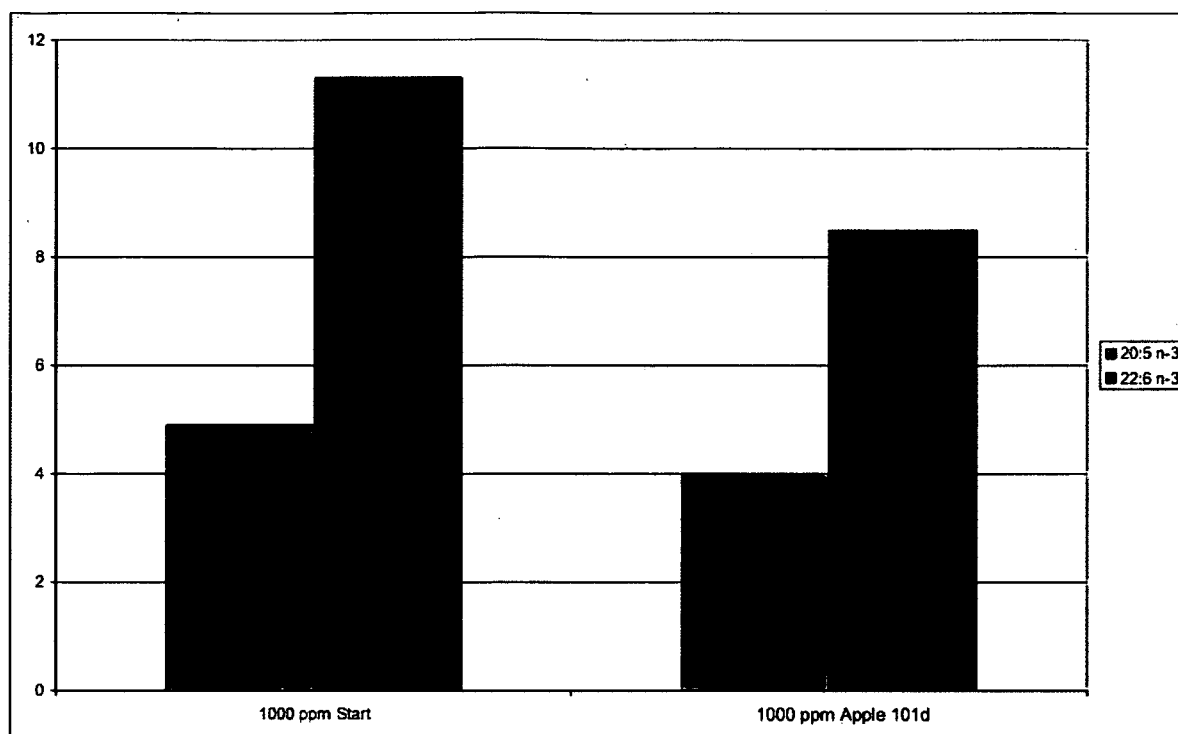


Figure 11

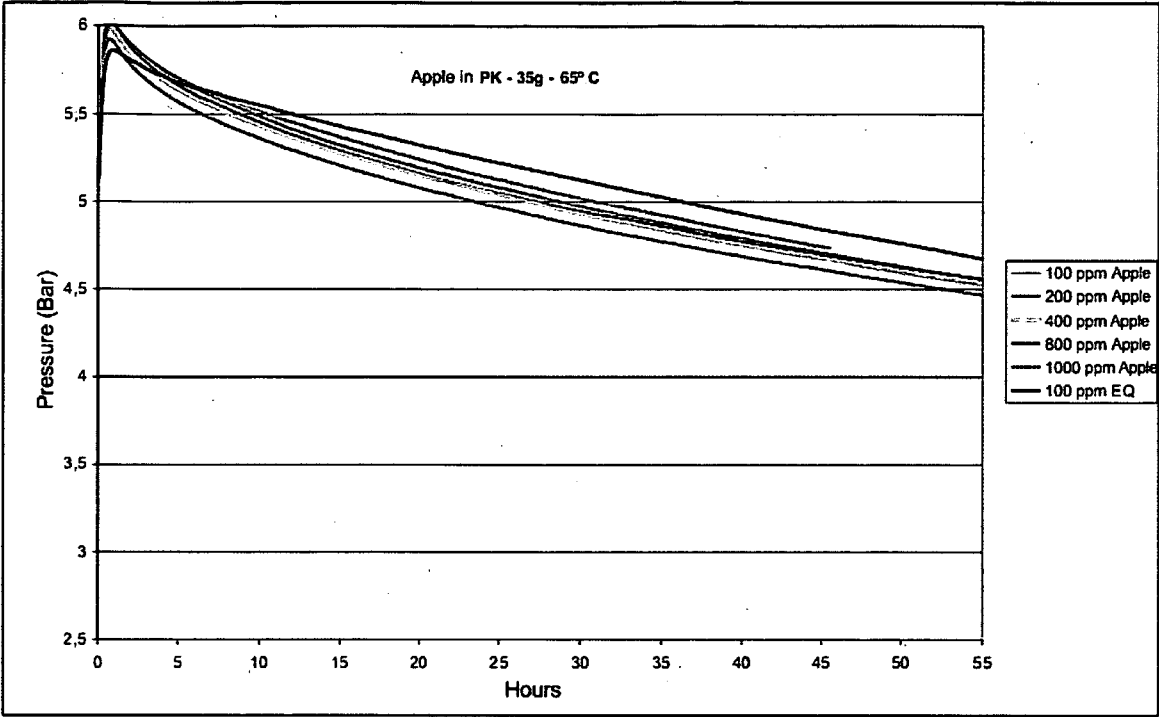


Figure 12

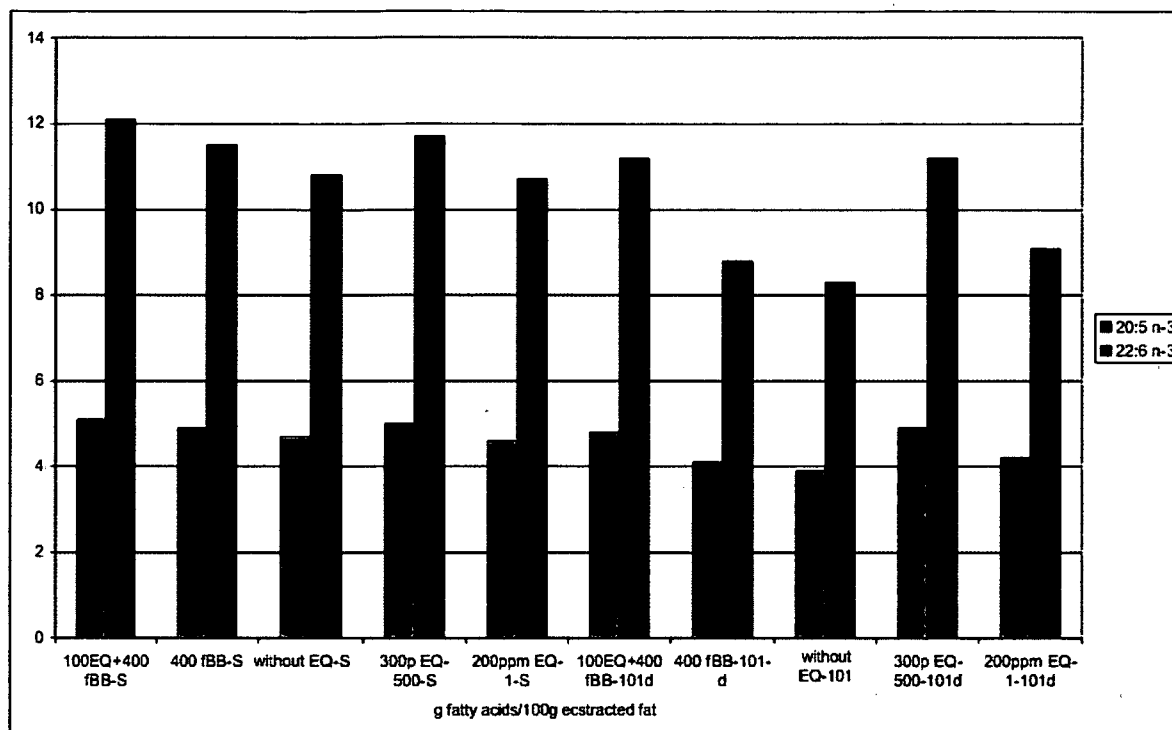
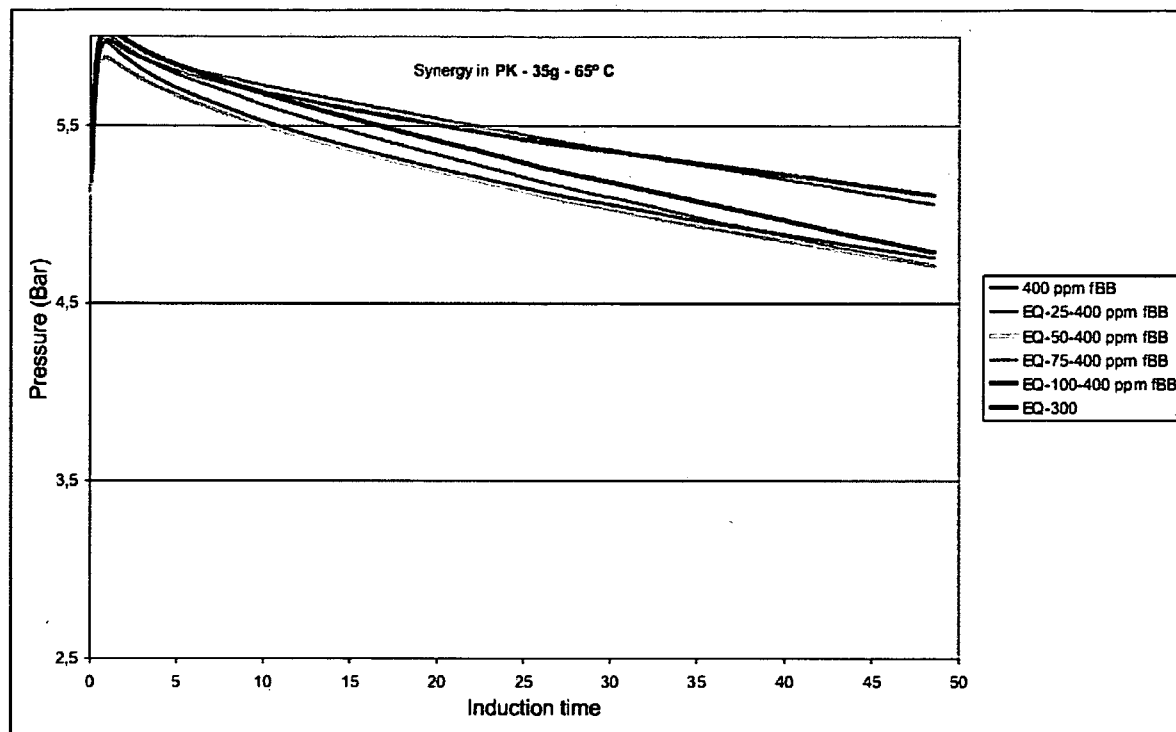


Figure 13



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO2010/000323

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:A23D, A23L, C09K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, WPI data, BIOSIS, EMBASE, MEDLINE, PUBCHEM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Landbo A-K. et. al. 'Enzyme-Assisted Extraction of Antioxidative Phenols from Black Currant Press Residues (Ribes nigrum)' In: Journal of Agricultural and Food Chemistry, 2001, vol 49, pp 3169-3177.; abstract; page 3170, column 1; table 8	1-37
A	-- Wolfe K L et. al. 'Apple peels as a value-added food ingredient.' In: Journal of Agricultural and Food Chemistry, 2003, vol 51, pp 1676-1683.; abstract; page 1681, column 1	1-37
A	-- US 20040234671 A1 (ECTOR BETTY J HOUSTON ET AL), 25 November 2004 (2004-11-25); paragraphs [0006]-[0007], [0017], [0026]	1-37
A	-- WO 03042133 A1 (HORTICULTURE & FOOD RES INST ET AL), 22 May 2003 (2003-05-22); page 15; claims 6-9	1-37
Y	-- WO 2009076776 A1 (CANADA NATURAL RESOURCES ET AL), 25 June 2009 (2009-06-25); paragraphs [0001]-[0002]; claims 1, 6, 7, 12-13, 17, 28; example 2	1-37
	--	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

26-11-2010

Name and mailing address of the ISA/SE

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO2010/000323

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1878753 A1 (NICHIREI FOODS INC ET AL), 16 January 2008 (2008-01-16); claims; experiment 1.2.2, experiment 1.4 --	1-37
A	US 6190716 B1 (GALBREATH JR SCOTT O), 20 February 2001 (2001-02-20); column 2, line 18 - column 2, line 67 -- -----	1-37

Continuation of: second sheet

International Patent Classification (IPC)

C09K 15/34 (2006.01)

A23D 9/06 (2006.01)

A23L 1/212 (2006.01)

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Use the application number as username. The password is **EIUBOWIVLO**.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO2010/000323

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **7-12, 23-28, 36**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Present claims 7-12, 23-28 and 36 relate to a method and a product defined by

.../...

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of: Box No. II

reference to the following desirable characteristics that the concentrate should have:

1. The amount of antioxidants in pressed fruit or berries. (claim 7)
2. The pressed fruit should stop the oxidation of different fatty acids. (claims 8-12 and 24-28)
3. The pressed fruit should decrease the amount of etoxyquin with a certain percent . (claim 23)
4. The amount of different antocyanidins in the pressed fruit or berries. (claim 36)

The claims cover all products and methods having these characteristics, whereas the application provides support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT for only a very limited number of such products and methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product and method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to method in claims 1-6, 13-22, 29-35 and 37.

Thence it follows that a reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability only can be established for those parts which have been searched.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/NO2010/000323

US	20040234671 A1	25/11/2004	NONE			
WO	03042133 A1	22/05/2003	CA	2466514 A1	22/05/2003	
			EP	1444177 A1	11/08/2004	
			NZ	515523 A	30/04/2004	
			US	20050095332 A1	05/05/2005	
WO	2009076776 A1	25/06/2009	NONE			
EP	1878753 A1	16/01/2008	BR	PI0609051 A2	17/02/2010	
			WO	2006090935 A1	31/08/2006	
US	6190716 B1	20/02/2001	NONE			