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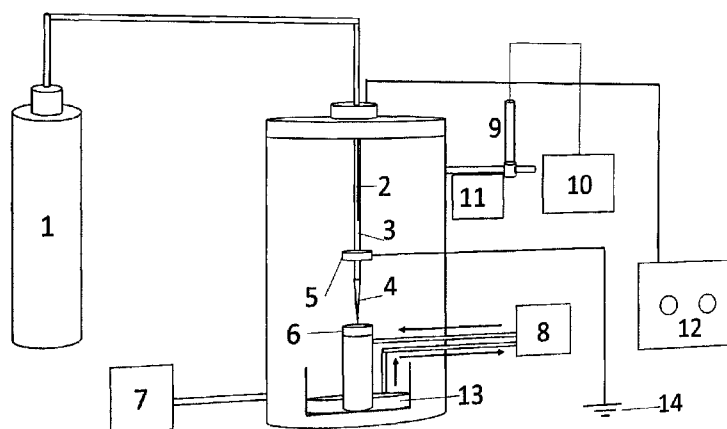
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(54) Title: NON-THERMAL PLASMA SYSTEM FOR ACCELERATED AGEING OF ORGANIC SAMPLES

Figure 1



(57) Abstract: Present invention in general concerns a system and an apparatus to accelerate oxidation chemistry of sample of organic products by non-thermal plasma based on variability due to the difference of the physico-chemical properties of the organic samples to be tested. More particularly the non-thermal plasma system or non-thermal plasma apparatus comprises at least one enclosure for connecting or removing sample holders so that the apparatus can comprise at the position under the NTP comprise different types of sample holders and preferably it comprises such enclosures to hold a plurality of sample holders. Preferably this sample holder is functionally connected with a motor so that separate sample holders can be moved under the non-thermal plasma (NTP) discharge and so that the sample position of the sample holder is controllable. This is system and an apparatus functionally connected with a volatile trap in such a way that a volatiles released from said the composition it is trapped. The sample holder can comprises an attachment means or an enclosure on top so that it can be covered by a NTP penetrable mesh. In a specific embodiment the trap is connected to or followed by a VOC analyser, for instance a VOC analyzer that comprises detectors of the group consisting of an electronic nose, FID, MS, UV, IR, TOF and SIFT-MS.



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NON-THERMAL PLASMA SYSTEM FOR ACCELERATED AGEING OF ORGANIC SAMPLES

FIELD OF THE INVENTION

5 Present invention in general concerns a system and an apparatus to accelerate oxidation chemistry of sample of organic products by non-thermal plasma based on variability due to the difference of the physico-chemical properties of the organic samples to be tested. More particularly the non-thermal plasma system or non-thermal plasma apparatus comprises at least one enclosure for connecting or removing sample holders so that the apparatus can comprise
10 at the position under the NTP comprise different types of sample holders and preferably it comprises such enclosures to hold a plurality of sample holders. Preferably this sample holder is functionally connected with a motor so that separate sample holders can be moved under the non-thermal plasma (NTP) discharge and so that the sample position of the sample holder is controllable. This is system and an apparatus functionally connected with a volatile trap in such
15 a way that a volatiles released from said the composition it is trapped. The sample holder can comprises an attachment means or an enclosure on top so that it can be covered by a NTP penetrable mesh. In a specific embodiment the trap is connected to or followed by a VOC analyzer, for instance a VOC analyzer that comprises detectors of the group consisting of an electronic nose, FID, MS, UV, IR, TOF and SIFT-MS.

20

BACKGROUND OF THE INVENTION

These is a need for faster, better controlled non-thermal oxidation of organic samples to be sensed for the in the formation of a unique set of oxidation products. This unique set of
25 oxidation products produced contain information to characterize, describe and product sample quality.

The sample position of the sample holder is controllable such that when operational the sample position can be placed in or under the plasma discharge. The plasma discharge originates an
30 controlled set of highly reactive species (radiation, radicals, excited species, ions) that might intensify/accelerate chemical oxidation reactions at ambient conditions. The latter circumstances are essential in order to guarantee representative chemical mechanisms.

The apparatus is useful to analyze types samples with different physico-chemical properties.

Moreover the apparatus of present invention allows the differentiation of tested samples as consequence of exposure to plasma discharge which is adaptable in a controllable manner. Present invention enables the evaluation of quality, oxidative stability, authenticity, purity and aroma stability of the introduced sample by performing post-hoc analyses on the sample or by
5 analysing the aforementioned produced VOC (Volatile Organic Compounds) compounds during/after pre-treatment by the invention. This allows the treatment of a large variety of samples both food as non-food matrices that are prone to oxidation resulting in the production of an unique set of volatile organic compounds. For instance food ingredients and food products; lipids, fatty acids, proteins, peptides, amino acids, carbohydrates, antioxidants, etc.
10 and non-food ingredients and products; polymers, paints, colouring agents, glues, building products, etc.

SUMMARY OF THE INVENTION

15 The present invention solves the problems of the other tests to accelerate oxidation chemistry by non-thermal plasma of sample of organic and inorganic products that are characterized by variability of oxidation chemistry due to the difference of the physico-chemical properties of the organic and inorganic samples to be tested. The invention enables a faster, better controlled non-thermal oxidation of organic samples to be sensed for the in the formation of a unique set
20 of oxidation products. This unique set of oxidation products produced contain information to characterize, classify, describe and product sample quality.

In accordance with the purpose of the invention, as embodied and broadly described herein, the invention is broadly drawn to a technical solution whereby the sample position of the sample holder is controllable such that when operational the sample position can be placed in or under
25 the plasma discharge. The plasma discharge originates from an controlled set of highly reactive species (radiation, radicals, excited species, ions) that might intensify/accelerate chemical oxidation reactions at ambient conditions. The latter circumstances are essential in order to guarantee representative chemical mechanisms.

In one aspect of the invention, this provides an apparatus that is useful to analyze types samples
30 with different physico-chemical properties. The apparatus of present invention allows differentiation of plasma discharge adaptable in a controllable manner. Present invention enables the evaluation of quality, oxidative stability, authenticity, purity and aroma stability of the introduced sample by performing post-hoc analyses on the sample or by analyzing the aforementioned produced VOC compounds during/after pre-treatment by the invention. This

allows the treatment of a large variety of samples both food as non-food matrices that are prone to oxidation resulting in the production of an unique set of volatile organic compounds. For instance food ingredients and food products; lipids, fatty acids, proteins, peptides, amino acids, carbohydrates, antioxidants, etc. and non-food ingredients and products; polymers, paints, colouring agents, glues, building products, etc.

The present invention provides an apparatus to accelerate oxidation in a sample of organic products by non-thermal plasma, the apparatus comprising a reaction chamber equipped with a build-in plasma reactor and a build in sample holder comprised in the reaction chamber, whereby the reaction chamber is adapted for airtight closure (by seal, an air lock) so that it can operate under atmospheric conditions or under a selected gas atmospheres. Some of the techniques described above may be embodied as the apparatus comprises a reaction chamber that is 1) connected or connectable to a gas fluid pump for extracting said fluid, such as air, from the reaction chamber and whereby the reaction chamber is 2) connected to at least one gas container for introduction of the specific gas, for example Helium or Argon. When operational the gas pump and the gas container are for replacing ambient air in said the reaction chamber by a gas of choice. This embodiment of the invention advantageously comprises that the sample position of the sample holder is controllable such that when operational the sample position can be placed in or under the plasma discharge. This can be further embodied in that the non-thermal plasma reactor is installed inside the airtight reaction chamber of aid apparatus and further that the non-thermal plasma (NTP) reactor to generate oxidative reactive species on a non-thermal way is any one of the group consisting of a corona discharge reactor, dielectric barrier discharger (DBD), plasma jet discharger (one or two electrodes are covered with dielectric barrier material) and glow discharger. The energy source for non-this thermal plasma can be DBD (for instance 1 kHz to 1MHz) or RF (for instance in a range of 10 to 15 KHz and preferably about 14 MHz). In a specific embodiment of the invention, the electrodes non-thermal plasma reactor have any one of the following shapes pin shaped, hollow pin, conic or flat. Hereby the electrodes in a specific embodiment are at a distance of 0.1 mm to 1 mm in between. Such electrodes preferably comprise Tungsten, stainless steel, copper, copper alloy. According to an embodiment of present invention the apparatus comprises a gas container is connected or connectable to the reaction chamber with specific features for optimal functioning such as the connection between the gas container and the reaction chamber comprises a fluid controller to control the gas flow, the gas container outlet or the reaction chamber inlet comprises a fluid controller to control the gas flow to sustain a constant plasma discharge can be varied between 0.001 sLm up to 5 sLm (sLm = standard litres per minute), the gas container

when partially or totally filled comprises a gas of the group consisting of pure argon, pure helium, argon:air mixture, argon: helium mixture, Ar/O₂, He/O₂, Ar/H₂O, He/H₂O and/or the connection between the gas container and the reaction chamber is a high-pressure tubing.

In a further embodiment of the invention, the apparatus of present invention is characterized in that said apparatus is operational in two modi operandi: a) as a stand-alone unit to pre-treat introduced sample matrices or b) hyphenated with a VOC (Volatile Organic Compounds) analyzer.

In one embodiment of the invention, the above described apparatus comprises a liquid pump to so that a liquid sample can be pumped through a sintered glass disk into the sample holder position under the NTP. Hereby the liquid sample introduction can comprise a sintered glass fountain.

According to an embodiment of the present invention there is also provided that the liquid samples holder can be position so that the NTP is close to the liquid sample or so that the NTP discharges inside the liquid sample. Solid materials can be fixated in the sample holder for NTP exposure. Hereby the solid sample holder can be position so that the NTP is close to the solid sample or so that the NTP discharges on the solid sample. Such apparatus can be adapted for online evaluation of VOC formation by the reaction chamber connected with gas conduit so that when operational gas loaded with volatile oxidation products is hyphenated to a VOC analyzer on the gas conduit or at the outlet of gas conduit.

In yet a specific embodiment the sample holder is functionally connected with a volatile trap in such a way that a volatiles released from said the composition it is trapped. Hereby the apparatus can be adapted for online evaluation of VOC formation by the reaction chamber connected with gas conduit so that when operational gas loaded with volatile oxidation products is hyphenated to a VOC trap at ambient conditions or a cooled VOC trap in such a way that volatiles released from a sample under NTP treatment are trapped. A suitable cooled trap is a Peltier cooled trap, a liquid/gas cooled trap, or a cryo trap (for instance a liquid nitrogen cooled trap). Furthermore in a specific embodiment the cooled trap comprises a tube containing sorbent material (e.g. tenax, Silica Gel, activated charcoal) or bubbled through ice-cooled water sample. It is also desirable to that the cooled trap is connected to or followed by a VOC analyser, for instance a VOC analyzer that comprises detectors of the group consisting of an electronic nose, FID, MS, UV, IR, TOF and SIFT-MS. Other preferred features of the apparatus of present invention are that the apparatus has at least one enclosure for connecting or removing the sample holder.

Another preferred further embodiment is that the sample holder is connectable and removable by and enclosure in the reaction chamber under the non-thermal plasma reactor, the enclosure is adaptable or removable so that the apparatus can comprise at the position under the NTP comprise different types of sample holders such as a fountain system holder, a solids/liquid sample holder, a cone samples holder, a cube sample holder, a cylindrical sample holder, the apparatus comprises an enclosure to fix a mesh above sample holder such mesh being NTP penetrable, the apparatus comprises a sample holder that comprises an attachment means or an enclosure on top so that it can be covered by a NTP penetrable mesh, the apparatus comprises an enclosures to hold a plurality of sample holders functionally connected with a motor so that separate sample holders can be moved under the NTP, the apparatus comprises at least one sampler holder with a volume in a range from 10 μ L to 10 000 ml.

This apparatus of present invention can be used in conjunction with generating accelerated ageing in said organic sample or in conjunction with generating accelerated oxidation chemistry in said organic sample.

Present invention concerns an apparatus to accelerate oxidation by non-thermal plasma in a sample comprising an organic product, the apparatus comprising a reaction chamber equipped with a build-in plasma reactor and a build in sample holder comprised in the reaction chamber, whereby the reaction chamber is adapted for airtight closure (by seal, an air lock) so that it can operate or under atmospheric conditions or under a selected gas atmospheres and whereby the sample holder is functionally connected or connectable with a volatile trap in such a way that a volatiles released from said the composition it is trapped.

In yet another embodiment the invention concerns an apparatus to accelerate oxidation by non-thermal plasma in a sample comprising an organic product, the apparatus comprising a reaction chamber equipped with a build-in plasma reactor and a build in sample holder comprised in the reaction chamber, whereby the reaction chamber is adapted for airtight closure (by seal, an air lock) so that it can operate or under atmospheric conditions or under a selected gas atmospheres and whereby the reaction chamber is connected with gas conduit so that when operational gas loaded with volatile oxidation products is hyphenated to the volatile trap in such a way that volatiles released from a sample under non-thermal plasma (NTP) treatment is trapped.

This volatile trap can be a cooled trap, for instance a cryo trap for instance a liquid nitrogen

cooled trap. This volatile trap can comprises a tube containing sorbent material, for instance tenax, silica gel or activated charcoal or it comprises an ice-cooled water sample for the volatile organic compounds to be guided or bubbled into. In a particular embodiment the cooled trap is connected or followed by a volatile organic compounds (VOC) analyzer.

5

Present invention also concerns in a particular embodiment an apparatus to accelerate oxidation by non-thermal plasma in a sample comprising an organic product, the apparatus comprising a reaction chamber equipped with a build-in plasma reactor and a build in sample holder comprised in the reaction chamber, whereby the reaction chamber is adapted for airtight closure (by seal, an air lock) so that it can operate or under atmospheric conditions or under a selected gas atmospheres and whereby the apparatus has at least one enclosure for connecting or removing the sample holder. A particular feature is whereby the enclosure is adaptable or removable so that the apparatus can comprise at the position under the NTP different types of sample holders such as a fountain system holder, a solids/liquid sample holder, a cone samples holder, a cube sample holder, a cylindrical sample holder.

15

According to one aspect of the invention the above described apparatus comprises an enclosure to fix a mesh above sample holder such mesh being non-thermal plasma (NTP) penetrable. According to one aspect of the invention the above described apparatus comprises sample holder that comprises an attachment means or an enclosure on top so that it can be covered by a non-thermal plasma (NTP) penetrable mesh.

20

According to another aspect the apparatus according to the invention as described above is characterized by that the apparatus comprises a VOC analyzer comprising a detectors of the group consisting of an electronic nose, flame ionization detector (FID), mass spectrometry (MS), ultraviolet (UV), infrared (IR), time-of-flight mass spectrometer (TOF) and selective ion flow tube-mass spectrometry (SIFT-MS).

25

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the reaction chamber is 1) connected or connectable to a gas fluid pump for extracting said fluid, such as air, from the reaction chamber and whereby the reaction chamber is 2) connected to at least one gas container for introduction of the specific gas, for example helium or argon.

30

According to another aspect the apparatus according to the invention as embodied in the

description above is in a particular embodiment characterized in that when operational the gas pump and the gas container are for replacing ambient air in said the reaction chamber by a gas of choice.

5 According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the sample position of the sample holder is controllable such that when operational the sample position can be placed in or under the plasma discharge.

10 According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the non-thermal plasma reactor is installed inside the airtight reaction chamber of said apparatus.

According to another aspect the apparatus according to the invention as embodied in the
15 description above is in a particular embodiment characterized in that the non-thermal plasma (NTP) reactor to generate oxidative reactive species on a non-thermal way is any one of the group consisting of a corona discharge reactor, dielectric barrier discharger (DBD), plasma jet discharger (one or two electrodes are covered with dielectric barrier material) and glow discharger.

20 According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the energy source for non-thermal plasma is dielectric barrier discharge or radio frequency discharge.

25 According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the electrodes non-thermal plasma reactor have any one of the following shapes pin shaped, hollow pin, conic or flat.

According to another aspect the apparatus according to the invention as embodied in the
30 description above is in a particular embodiment characterized in that the electrodes are at a distance of 0.1 mm to 1 mm in between.

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the materials of the

electrodes comprise Tungsten, stainless steel, copper, copper alloy.

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the gas container is
5 connected or connectable to the reaction chamber.

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the connection between the gas container and the reaction chamber comprises a fluid controller to control the gas flow.
10

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the gas container outlet or the reaction chamber inlet comprises a fluid controller to control the gas flow to sustain a constant plasma discharge can be varied between 0.001 sLm up to 5 sLm (sLm = standard liters
15 per minute).

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the gas container when partially or totally filled comprises a gas of the group consisting of pure argon, pure helium, argon:air mixture, argon: helium mixture, Ar/O₂, He/O₂, Ar/H₂O, He/H₂O.
20

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that said apparatus is operational in two modi operandi: a) as a stand-alone unit to pre-treat introduced sample matrices or b) hyphenated with a VOC (Volatile Organic Compounds) analyzer.
25

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the connection between the gas container and the reaction chamber is a high-pressure tubing.
30

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the apparatus comprises a liquid pump to so that a liquid sample can be pumped through a sintered glass disk into the sample holder position under the non-thermal plasma (NTP).

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the liquid sample introduction comprises a sintered glass fountain.

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According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the liquid samples holder can be position so that the NTP is close to the liquid sample or so that the NTP discharges inside the liquid sample.

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According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that a solid sample holder can be position so that the NTP is close to the solid sample or so that the NTP discharges on the solid sample.

15

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that said apparatus is adapted for online evaluation of VOC formation by the reaction chamber connected with gas conduit so that when operational gas loaded with volatile oxidation products is hyphenated to a VOC analyzer on the gas conduit or at the outlet of gas conduit.

20

According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that the sample holder is connectable and removable by and enclosure in the reaction chamber under the non-thermal plasma reactor.

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According to another aspect the apparatus according to the invention as embodied in the description above is in a particular embodiment characterized in that it comprises enclosures to hold a plurality of sample holders functionally connected with a motor so that separate sample holders can be moved under the NTP.

30

Further scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it is understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the

invention will become apparent to those skilled in the art from this detailed description. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

5 **Detailed Description**

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The following detailed description of the invention refers to the accompanying drawings. The same reference numbers in different drawings identify the same or similar elements. Also, the following detailed description does not limit the invention. Instead, the scope of the invention
10 is defined by the appended claims and equivalents thereof.

The following detailed description of the invention refers to the accompanying drawings. The same reference numbers in different drawings identify the same or similar elements. Also, the following detailed description does not limit the invention. Instead, the scope of the invention is defined by the appended claims and equivalents thereof.

15 Several documents are cited throughout the text of this specification. Each of the documents herein (including any manufacturer's specifications, instructions etc.) are hereby incorporated by reference; however, there is no admission that any document cited is indeed prior art of the present invention.

The present invention will be described with respect to particular embodiments and with
20 reference to certain drawings but the invention is not limited thereto but only by the claims. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn to scale for illustrative purposes. The dimensions and the relative dimensions do not correspond to actual reductions to practice of the invention.

25 Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

30 Moreover, the terms top, bottom, over, under and the like in the description and the claims are used for descriptive purposes and not necessarily for describing relative positions. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other orientations than described or illustrated herein.

It is to be noticed that the term "comprising", used in the claims, should not be interpreted as being restricted to the means listed thereafter; it does not exclude other elements or steps. It is thus to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more other features, integers, steps or components, or groups thereof. Thus, the scope of the expression "a device comprising means A and B" should not be limited to the devices consisting only of components A and B. It means that with respect to the present invention, the only relevant components of the device are A and B.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to one of ordinary skill in the art from this disclosure, in one or more embodiments.

Similarly it is appreciated that in the description of exemplary embodiments of the invention, various features of the invention are sometimes grouped together in a single embodiment, figure, or description thereof for the purpose of streamlining the disclosure and aiding the understanding of one or more of the various inventive aspects. This method of disclosure, however, is not to be interpreted as reflecting an intention that the claimed invention requires more features than are expressly recited in each claim. Rather, as the following claims reflect, inventive aspects lie in less than all features of a single foregoing disclosed embodiment. Thus, the claims following the detailed description are hereby expressly incorporated into this detailed description, with each claim standing on its own as a separate embodiment of this invention.

Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the following claims, any of the claimed embodiments can be used in any combination.

In the description provided herein, numerous specific details are set forth. However, it is understood that embodiments of the invention may be practiced without these specific details. In other instances, well-known methods, structures and techniques have not been shown in detail in order not to obscure an understanding of this description.

Other embodiments of the invention will be apparent to those skilled in the art from

consideration of the specification and practice of the invention disclosed herein.

It is intended that the specification and examples be considered as exemplary only.

Each and every claim is incorporated into the specification as an embodiment of the present invention. Thus, the claims are part of the description and are a further description and are in addition to the preferred embodiments of the present invention.

Each of the claims set out a particular embodiment of the invention.

The following terms are provided solely to aid in the understanding of the invention.

Non thermal plasma technology can be used for oxidation tests on any organic and inorganic matrix that can be oxidized, such as for example fish oil. An example of fish oil samples that we used for menhaden fish oil (Sigma Aldrich, Diegem (Belgium)) was purchased and stored at -80°C to prevent further oxidation. For each test, fish oil samples were used, either pure or enriched with an antioxidant (100 µg/g and/or 1000 µg/g α -Tocopherol (Sigma Aldrich)). The fatty acid composition of the Menhaden fish oil was provided by Sigma Aldrich and is expressed in percentage. For the used fish oil, the following initial typical fatty acid composition is applicable; 30.4 % saturated fatty acids (7.94 % C14:0, 15.1 % C16:0, 3.8 % C18:0), 26.7 % mono-unsaturated fatty acids (10.5 % C16:1, 14.5 % C18:1, 1.3 % C20:1, 0.4 % C22:1) and 34.2 % poly-unsaturated fatty acids (2.2 % C18:2, 1.5 % C18:3, 2.8 % C18:4, 1.1 % C20:4, 13.2 % C20:5, 4.9 % C22:5, 8.6 % C22:6). Non thermal plasma can be used on these samples for oxidation test. In particular for natural aging . For instance according to the state of the art for natural aging (reference) 100 grams of pure fish oil and 100 grams of enriched (1000 µg/g α -tocopherol) fish oil was put in an Erlenmeyer and kept in the dark at ambient conditions for 11 weeks. Every week 3 g of oil was sampled and stored at -80°C to prevent further oxidation.. Natural storage tests take a lot of time!!! The NTP of present invention does this in a much faster manner.

An example of thermal accelerated oxidation test of the art is as follows. Thermal treatment of the fish oil was performed at 100 °C for 6 hours, based on the widely used Rancimat test (Lutterodt, Slavin, Whent, Turner, & Yu, 2011). In each experiment 50 g of fish oil was put in a glass flask and heated to the desired temperature by placing it in a temperature controlled oven. Air was bubbled for 6 hours through the sample (using a sintered glass disk for maximum contact with the oil) at a flow rate of 1.0 L/min. The oil was continuously stirred by the air stream passing through the sample, creating an optimum transfer of oxygen to the heated oil. After passing through the oil, the air bubbled through an ice-cooled water sample of 100 g in order to capture secondary volatile lipid oxidation compounds. After thermal treatment, 0.5 g

of the water sample was transferred into a 20 mL headspace vial and sealed using an inert Teflon septum. Afterwards, the same treatment was applied to oil containing 1000 µg/g α-tocopherol. The NTP of present invention is suitable for thermal accelerated oxidation test.

- 5 This is a highly described description of a possible NTP reactor that has been used to develop/proof the invention. In this section plasma chemistry but also details of a possible (already available) NTP reactor is given.

Dielectric barrier discharge plasma is suitable for accelerated oxidation treatment. DBD plasma
10 operating with Ar/O₂ mixture as a feed gas in ambient air can be considered as a source of a broad range of active species. The species generated in the active zone of the discharge located in between electrodes can be divided in (listed according to increasing reactivity): charged particles (electrons, positive and negative ions); neutral excited states of Ar (metastables, resonance states and electron excited states); UV and VUV photons (appearing due to excimer
15 radiation, OH and NO bands emission); oxygenated species including O₃, O₂ singlet, and O. The production mechanisms of different excited species have been intensively studied in the last decade worldwide. In the research of Van Gils, Hofmann, Boekema, Brandenburg, & Bruggeman (2013) and Reuter et al. (2012) production of VUV and UV radiation in plasma of Ar using a slightly higher power of 20 W has been studied and absolute VUV radiance has been
20 estimated around 2-3 mWmm⁻²sr⁻¹. Such low amount of VUV/UV photons cannot explain observed chemical changes during oil treatments. Therefore the effect of UV radiation can be excluded. Considering the low ionization degree of our plasma with an electron density of about 1.5x10¹³ cm⁻³ and taking into account dissociative electron-ion recombination which has a typical rate of 10⁻¹³ m³ s⁻¹, the actual density of charged particles that reaches the treated surface
25 in the far afterglow is 2-3 orders of magnitude lower than the density of the charged particles in the active zone. The charged particles concentration of about 10⁻¹⁰ cm⁻³ cannot considerably affect chemical reactions in the liquid phase during our experiments. Active species of Ar, especially those with long lifetime as metastable and resonance states, can reach the surface of the treated oil. Ar excited states cannot directly oxidize the oil but can initiate formation of free
30 radicals in the liquid. This process has been checked in an independent experiment of Van Durme, Nikiforov, Vandamme, Leys, & De Winne (2014) in which Ar plasma jet has been used for olive oil treatment. It was shown that the formation of oxidative products in oil under action of a pure Ar plasma jet is very low, even after 60 minutes of plasma treatment. Considering the above mentioned results, the effect of plasma treatment of liquid samples can be solely

attributed to oxygenated species including mainly O_3 , O_2 singlet, and atomic O .

25 grams of fish oil was put in a glass container. The oil was pumped through a sintered glass disk, which prevented the oil from being blown away during the NTP-treatment and increased the contact of the plasma jet and the oil. Sample losses were determined by weighing the sample before and after treatment. Less than 3% of sample was lost during 60 minutes of NTP treatment. Previous tests indicated that a direct treatment of the oil surface without a sintered glass disk led to an insufficient contact of the plasma with the oil. Secondly the oil would gush, leading to contamination of the quartz tube and eventually inhibiting the formation of a stable plasma jet. The plasma jet (Fig 1.b) was placed above the sintered glass disk, spreading over the oil surface. The distance between the capillary quartz tube and the sintered glass disk was 5 mm. Exposure times of 60 minutes were applied for plasma treatment. The plasma jet comprises a tungsten rod (energetic electrode) with a sharp tip, inserted in a quartz capillary with 1.3 mm inner diameter. The tungsten rod and quartz capillary together are centered inside a grounded aluminum tube (ground electrode). Alternating peak to peak voltage of 6 kV is applied to the tungsten rod by a 50 kHz power supply (Bayerle, Germany). Gas is fed into the plasma jet through two separated lines each controlled by a mass flow controller (Bronckhorst, Belgium). For the experimental configuration used in this study, a stable discharge was obtained when the voltage input was fixed at 6.00 kV (peak to peak) and current of 128 mA while maintaining an Argon gas flow rate of 2.00 slm (standard liters per minute). The Argon stream was doped with oxygen gas (0.6 %) in order to create the abovementioned oxidative species and eventually induce lipid oxidation, while maintaining the treated oil sample at ambient temperatures. Atomic oxygen concentration was measured using spectroscopy. More specific, an Ocean Optics s2000 spectrometer with resolution of 1.5 nm has been used for emission spectrometry of the plasma jet. Sensitivity of the spectrometer has been corrected with the use of a NIST calibrated Oriel model 65355 spectral lamp. Adding 0.6% of oxygen led to a total atomic oxygen concentration of $7.21 \times 10^{17} \text{ cm}^{-3}$.

It has to be noted that the measurement of singlet delta oxygen (SDO) molecules in the plasma jet is a technically challenging task due to the small size of the jet and a correspondingly low absorption signal. Among available results, most of the experiential studies of the singlet oxygen production have been carried out in conditions similar to those of our plasma jet but for He/O_2 mixtures by means of IR absorption. In the study of Sarani, Nikiforov, & Leys, (2010) the SDO absolute density was estimated to be around $6 \times 10^{15} \text{ cm}^{-3}$ for RF and DBD jets in an optimal He/O_2 mixture. Similar values in the order of 10^{15} cm^{-3} were obtained in the study of Lu & Wu (2013) for a low power plasma jet operating in ambient air. A density of 1.7×10^{15}

cm^{-3} of O_2 ($a^1\Delta_g$) was found in a microplasma jet operating in $\text{He}+2\% \text{O}_2$ (Sousa, Douat, Bauville, Fleury, & Puech, 2013). These experimental results have also been confirmed by numerical simulations where the SDO density was estimated at 10^{15}cm^{-3} in the He plasma jet. In recent work SDO densities were also estimated in an Ar plasma jet by a numerical study.

- 5 The authors have found that up to 1 cm away from the nozzle the O_2 ($a^1\Delta_g$) concentration is about $0.7 \times 10^{15} \text{cm}^{-3}$ and comparable with the density of atomic oxygen. They found that O_2 ($a^1\Delta_g$) initiated chemistry starts to be important only in the very far effluent, as its internal energy is rather low (0.98eV) compared with OH, Ar excited states and atomic O.

- For chemical analysis of volatile lipid oxidation products VOC analytical technique can be combined with the invention (HS-SPME-GC-MS). Isolation of the volatiles originated from lipid oxidation, was performed with an autosampler (MultiPurpose Sampler® or MPS®, GERSTEL®, Mülheim am der Ruhr, Germany), equipped with a headspace-solid phase microextraction unit. Solid-phase microextraction combined with one dimensional gas chromatography-mass spectrometry has been applied in many food related researches and already proved to be a sensitive and reliable methodology for the evaluation of volatile lipid oxidation products (Van Durme et al., 2014). Based on experiments (§3.1) the following sample preparation conditions were selected: 0.5 g of fish oil sample or water sample (§2.2.1) was hermetically sealed in brown 20 mL vials to be incubated 30 min. Next, the headspace was extracted at 60°C on a well-conditioned CAR/PDMS SPME fiber for another 30 minutes by means of a thermostatic agitator.

- A fully automated sample preparation unit (MultiPurpose Sampler® or MPS®, GERSTEL®, Mülheim an der Rur, Germany), combined with a 6890/5973 GC-MS system (Agilent Technologies®, Palo Alto, CA) was used for compound separation and identification. Helium was used as a carrier gas (1 mL/min). Injector and transfer lines were maintained at 250 °C and 280 °C, respectively. The total ion current (70 eV) was recorded in the mass range from 40 to 230 amu (scan mode) using a solvent delay of 2 min and a run time of 5 min. For GC-MS profiling, both a cross-linked methyl silicone column (HP-PONA), 50 m x 0.20 mm I.D., 0.5 µm film thickness (Agilent Technology®) and a ZB-WAX column, 30 m x 0.25 mm I.D., 0.25 µm film thickness (Phenomenex®) were used and programmed: 40 °C (5 min) to 160 °C at 3 °C/min, from 160 °C to 220 °C at 5 °C/min, held for 3 min. Identification of volatile organic compounds in the fish oil headspace was performed by comparison with the mass spectra of the Wiley® 275 library. Additionally, confirmation of identified compounds was done by determination of Kovats indices, determined after injection of a series of n-alkane homologues using the analytical configuration as described above. Thirdly, some authentic

reference standards were injected to confirm the identity of some important volatiles. Concentration of identified oxidation products were expressed semi-quantitatively, using an internal standard, 4-Hydroxyl-4-methyl-2-pentanone (10 μ L, 0.309 μ g/ μ L). All samples were measured in triplicate (n=3).

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Identification of odor active volatile oxidation markers is useful to analyze naturally aged fish

oil. In the following section, the naturally aged fish oil was evaluated over a period of 11 weeks

by identifying and quantifying volatile organic compounds in the headspace of the matrix. The

goal is to profile the aroma compounds in function of storage time and to identify a number of

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volatiles that are clear markers for lipid oxidative phenomena in fish oil. Although this

approach, using secondary volatiles to evaluate the lipid oxidation progress, is most realistic,

today most researchers still focus on measuring primary oxidation products by means of

peroxide value (PV). Secondary oxidation products are often evaluated by the thiobarbituric

acid reactive substances (TBARS) or the p-anisidine value (AV). Research papers in which

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volatiles are measured typically select hexanal as a typical lipid oxidation marker (Panseri,

Soncin, Maria, & Antonio, 2011). In fish oil however, hexanal is not a typical lipid oxidation

marker. Other oxidation products such as 1-penten-3-one (pungent green odor), Z-4-heptenal

(fishy odor), (E,E)-2,4-heptadienal (fatty, rancid odor), (E,Z)-2,6-nonadienal (cucumber odor)

and 1-octen-3-ol (mushroom odor) have been characterized as very potent odorants,

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contributing to the unpleasant rancid and fishy off-flavor. For this study, different solid-phase

microextraction (SPME) fibers were compared (CAR/PDMS, PDMS, CAR/DVB/PDMS) at

60 °C and an extraction time of 30 min. The most effective fiber type proved to be CAR/PDMS.

Using the selected fiber type (CAR/PDMS), extractions were performed at 40, 60, 80°C for 15,

30, 45 min. It was observed that a 30 minute extraction time was optimal, when preceded by

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incubating the sample for 30 min at 60 °C. Naturally aged fish oil was used for this

optimization. Table 1 represents semi-quantitatively determined concentrations of volatile

compounds present in fresh and naturally aged fish oil samples. In total 55 volatiles were

identified of which the aldehydes proved to be the most dominant, followed by hydrocarbons

and ketones. While in fresh fish oil a total volatile organic compound (VOC) concentration of

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*1.64*10³ μ g/g was already measured, a significant increase in VOC variety and concentration*

*was observed after 11 weeks of storage in ambient and dark conditions (3.82*10³ μ g/g). It is*

generally known that in this matrix practically no enzymatic lipid oxidation or other microbial

or fermentative processes can occur. Since enzymes are present in the watery phase of a

biological system, amounts of enzymes in the extracted oil are considered negligible.

Therefore, these observations can only be explained by lipid auto-oxidation, typically resulting in volatiles such as aldehydes (2-propenal, propanal, pentanal, heptanal, (E,E)-2,4-heptadienal and (E,E)-2,4-octadienal), ketones (1-octen-3-one, 3,5-octadien-2-one, 2-nonanone) and several hydrocarbons (tridecane, pentadecane). The lipid oxidation mechanism is initiated by free radicals which abstract a hydrogen atom at carbon atoms adjacent to a double bond. Triplet oxygen reacts with these lipid radicals leading to lipid peroxides formation. Further propagation reactions include hydroperoxide formation and β -scissions eventually resulting in the formation of secondary lipid oxidation volatiles. Reaction mechanism pathways of these oxidation volatiles are well described in literature (Frankel, 1984). Above mentioned results illustrate that HS-SPME-GC-MS is a sensitive, reproducible and relevant analytical technique to study oxidation phenomena in fish oil, hence this approach will be also used when studying lipid oxidation chemistry in both thermal and non-thermal plasma based lipid oxidation (§3.3). From Table 1 it can be derived that formic acid, 1-penten-3-ol, propenal, (E)-2-pentenal, heptanal, (E)-2-heptenal, (E,E)-2,4-heptadienal, (E,E)-2,4-octadienal, (E)-2-nonenal and (E)-2-decenal strongly increased during natural storage, making them important lipid oxidation products. Since it is well described that oxidized fish oil develops important off-aromas, it is of high importance to consider Odor Activity Values (OAV) when studying lipid oxidation. OAV values are calculated by dividing the specific headspace concentration by the corresponding odor threshold value. For the naturally aged oil most odor active lipid oxidation compounds proved to be 1-octen-3-one (14.40 $\mu\text{g/g}$, $\text{OAV} = 2.9 \cdot 10^6$), (E,Z)-2,6-nonadienal (24.99 $\mu\text{g/g}$, $\text{OAV} = 2.5 \cdot 10^6$), (E)-2-nonenal (51.74 $\mu\text{g/g}$, $\text{OAV} = 5.2 \cdot 10^5$), (E,E)-2,4-decadienal (17.87 $\mu\text{g/g}$, $\text{OAV} = 2.6 \cdot 10^5$), (E)-2-decenal (31.95 $\mu\text{g/g}$, $\text{OAV} = 1.1 \cdot 10^5$), 3,5-octadien-2-one (85.68 $\mu\text{g/g}$, $\text{OAV} = 7.1 \cdot 10^4$), 1-penten-3-one (43.13 $\mu\text{g/g}$, $\text{OAV} = 4.3 \cdot 10^4$), (E,E)-2,4-heptadienal (517.9 $\mu\text{g/g}$, $\text{OAV} = 3.5 \cdot 10^4$), 1-octen-3-ol (33.28 $\mu\text{g/g}$, $\text{OAV} = 3.3 \cdot 10^4$), (E)-2-octenal (55.23 $\mu\text{g/g}$, $\text{OAV} = 1.9 \cdot 10^4$), nonanal (16.76 $\mu\text{g/g}$, $\text{OAV} = 1.7 \cdot 10^4$), pentanal (25.28 $\mu\text{g/g}$, $\text{OAV} = 2.1 \cdot 10^3$) and propanal (38.99 $\mu\text{g/g}$, $\text{OAV} = 1.1 \cdot 10^3$). Using this approach, completed by literature study, enabled to select a list of the most important Lipid Oxidation Markers (LOMs) as summarized in Table 2. These LOMs were used in this paper to evaluate and compare both the thermal (§ 3.2) and non-thermal plasma (§ 3.3) based accelerated lipid oxidation methods.

Lipid oxidation marker assessment for evaluation of antioxidant effectiveness during the natural aging test

Figure 2a illustrates changes in headspace concentrations above fish oil samples for a number of the selected LOMs summarized in Table 2, more specific 2-propenal, (E)-2-pentenal, (E)-2-

decenal, 1-octen-3-ol and (E,E)-2,4-octadienal. In agreement with other studies (Horn, Nielsen, & Jacobsen, 2009) a clear anti-oxidative effect of adding 1000 µg/g α -tocopherol is visualized in Figure 2a, showing a reduced formation after 11 weeks for 2-propenal, (E)-2-pentenal, (E)-2-decenal, 1-octen-3-ol and (E,E)-2,4-octadienal. This result indicates that α -tocopherol and γ -tocopherol both have antioxidant properties when used in the conditions described earlier. Horn et al. (2009) determined a prooxidative effect of γ -tocopherol addition below 200 µg/g. This experiment has not been repeated in this work since this effect has already been well described.

Thermal Treatment generates VOC's. Based on VOC measurements of thermally treated fish oil, it could be concluded that some compounds identified in naturally aged fish oil could not be detected after the thermal treatment. This was for example the case for ethanol, acetaldehyde, 2-methyl-2-butenal, (E,E,E)-2,4,6-octatrienal, (E,E)-2,4-decadienal, 1-hydroxy-2-butanone and 5-ethyl-2(5H)-furanone.

Secondly the relative VOC composition after thermal treatment proved to be completely different compared to that measured in naturally aged fish oil. For example the relative class importance of aldehydes for naturally aged fish oil was 33%, while this was 82% for thermally oxidized fish oil. Figure 2b illustrates the formation of the earlier identified volatile lipid oxidation markers. Thirdly, the overall concentration range of the VOCs seems to be much higher after the thermal treatment compared to the natural aging process. Since 11 weeks of natural aging resulted in concentrations up to 35 µg/g for 1-octen-3-ol and 55 µg/g for (E)-2-pentenal, a thermal treatment of 6 hours resulted in concentrations for these compounds of respectively 550 µg/g and 1100 µg/g. Formation of 2-propenal, (E)-2-pentenal, (E)-2-decenal, 1-octen-3-ol and (E,E)-2,4-octadienal are presented in figure 2b.

Furthermore, in contrary to the results as measured during ambient storage test, the addition of 1000 µg/g α -tocopherol clearly resulted in a prooxidative effect during thermal exposure, leading to increased lipid oxidation products. Instead of working as a chain-breaking antioxidant preventing propagation of free radicals, the high temperature inverted these antioxidative properties of α -tocopherol into prooxidative effects.

Based on these results it can be concluded that the thermal accelerated lipid oxidation test insufficiently correlates with natural oxidation of fish oil. Besides the different composition and higher concentration of oxidation products, the addition of α -tocopherol (1000 µg/g) results in a prooxidative effect during the thermal treatment, while an antioxidative effect was observed at ambient temperature. Since the adverse effects of elevated temperatures have already clearly been proven during this study and in other research papers (Mancebo-Campos, Salvador, &

Fregapane, 2014), experiments with α -tocopherol enriched fish oil at 100 μ g/g were not performed.

The apparatus and system of present invention is to suitable to treat an organic matrix and any oxidizable matrix in order to produce volatiles to characterize said matrix or analyze its volatile fingerprint or to analyze or assess the quality, age or shelf life of said matrix. A suitable matrix for present invention is for instance a polyunsaturated fatty acids (PUFA) matrix. The enhanced incorporation of PUFA has become an important topic for the food industry due to their wide range of nutritional and health benefits for the end consumer. These positive effects have been described mainly for ω -3 and ω -6 PUFAs (Skall & Anne, 2008). Numerous epidemiological, clinical, animal and in situ experiments have shown health benefits due to an increased intake of ω -3 fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Studies revealed a including decreased risk of coronary heart disease, immune response disorders and mental illness, as well as benefits to infants and pregnant women. Sources containing high levels of these unsaturated fatty acids are nuts, vegetable oils, fish and soybeans. In the last years, increasing attention is given to new, sustainable sources of these PUFAs, such as microalgae or extracts of microalgae that can be integrated in a variety of foodstuffs (Barba, 2016).

Despite the many advantages of increasing the PUFA content in food matrices, a major issue is their high susceptibility to lipid oxidation. This oxidative phenomenon inevitably leads to loss of shelf-life, consumer acceptability, functionality, nutritional value, organoleptic properties and safety. The intensity of lipid oxidative deterioration of PUFA enriched foodstuffs depends on different factors; particularly the degree of unsaturation of fatty acids and the presence of external factors promoting oxidation, e.g. exposure to oxygen and light, metallic ions or high temperatures. The oxidative stability of each of these PUFAs is inversely proportional to the number of bis-allylic hydrogens in the molecule; therefore, EPA and DHA are even more easily oxidized compared to oleic acid, linoleic acid and linolenic acid (G. Delgado-Pando, S. Cofrades, C. Ruiz-Capillas, M. Triki, 2012).

There are few reports on accurate shelf-life tests for the evaluation of lipid oxidation in PUFA enriched food products that specifically focus on the organoleptic changes developing during storage. For food manufacturers it is of high importance to safeguard the initial nutritional and organoleptic characteristics during the shelf-life. In line with the abovementioned trend, the

development and improvement of methods to evaluate the oxidative stability of food products have received growing attention in the last years. Due to practical reasons, researchers have been especially focusing on accelerated shelf-life tests. Such techniques have great application possibilities in the study of lipid oxidation, oil stability, off-flavor formation chemistry, the prediction of possible intermediate formation and the impact of oxidation on the nutritional properties of food in a faster manner (Van Durme et al., 2014). Moreover, these techniques can also be used for the assessment of the functionality of synthetic and natural antioxidants in PUFA-enriched food products.

In practice, most of the accelerated oxidation techniques are based on increased temperatures (e.g. Swift test, Rancimat). Rancimat is the most widely used test for accelerated lipid oxidation. An oil sample is heated to the desired temperature while air is bubbled through at a constant flow rate. Next the air, loaded with the formed oxidation volatiles, is sent through a water sample in which the volatiles of the oil sample are transferred. After the experiment an oil matrix is left of which all formed oxidation products have been stripped. In this way a sensory evaluation of this accelerated 'aged' product is not possible. Secondly, outcomes of thermally-based techniques poorly correlate with realistic storage tests. This can be explained by the fact that the mechanism of lipid oxidation changes when temperatures exceed 60 °C (Mancebo-Campos, Fregapane, & Salvador, 2008). No marked success has ever been achieved in realistically predictioning organoleptic changes and/or shelf-life of edible fats and oils by such thermally based stability tests. Some studies in literature revealed that most accelerated tests are performed at temperatures of at least 100 °C (Farhoosh & Hoseini-Yazdi, 2013). Next to deviating lipid oxidation kinetics, other reactions such as polymerization, thermal degradation, cyclization, Maillard reactions, Strecker degradation, denaturation or oxygen depletion could occur at such high temperatures (Van Durme, Nikiforov, Vandamme, Leys, & De Winne, 2014). Secondly, these thermally based techniques remain relatively time-consuming (up to several days). Moreover, some antioxidants are thermally unstable, which leads to an under –or overestimation of their effect.

Abovementioned factors indicate that innovative accelerated oxidation techniques are required which operate at ambient temperatures and which are able to accelerate lipid oxidation processes in both a fast and reliable manner. Moreover, the development of an accelerated oxidation test enabling the user to perform a sensory analysis on the treated sample would be of great value for the food industry. In this paper, the applicability of Non-Thermal Plasma (NTP) will be investigated as a new innovative accelerated lipid oxidation test using fish oil as a case. NTP is generally described as the fourth state of matter and comprises reactive species

(atoms, ions, radicals), formed by dissociative electron attachment processes. Several applications of NTP have already been described in literature, such as removal of pollutants in water, medical applications surface treatments and gas emission treatments (Durme, Dewulf, Leys, & Langenhove, 2008). However, besides sanitation of food products (Baier et al., 2013) and first experiments on a commercial blend of vegetable oil (Van Durme, Nikiforov, Vandamme, Leys, & De Winne, 2014), no applications of NTP for the accelerated oxidation of lipids in food have been reported. The primary goal of this work is to investigate whether NTP treatment induces realistic lipid oxidation reactions in fish oil, and to what degree they correlate with natural lipid auto-oxidation. This was assessed by measuring and comparing the secondary volatile lipid oxidation products as markers for food ageing. Experiments were performed using Ar/O₂ plasma on fish oil as a reference material. These results are compared to thermally oxidized and naturally aged fish oil samples.

Olive oil is a highly desirable food product on the international market due to its unique organoleptic properties, its high monounsaturated fatty-acid content and antioxidant properties (Bendini et al., 2007). Safeguarding the quality of these olive oils is of utmost importance.

Much research is focusing on the aroma and flavor properties of olive oils. The organoleptic quality of olive oils is mostly assessed using trained taste panels or by means of headspace-solid phase microextraction (Romero, García-González, Aparicio-Ruiz, & Morales, 2015). Recently, (Uncu & Ozen, 2015) described a Fourier transform infrared spectroscopy method to determine quality parameters such as oxidative stability, color pigments, fatty acid profile and phenolic composition of olive oils. Next to the abovementioned focus on overall quality of fresh olive oil, increasing research attention is given to oxidative deterioration of olive oils. (Krichene et al., 2010) studied the stability of virgin olive oil and behavior of its natural antioxidants under medium temperature conditions. Equally important is the development of analytical techniques to verify the traceability of high-quality monovarietal olive oils. In order to verify the claimed botanical origin, multiple techniques have been developed such as Near Infrared (NIR) Spectroscopy, Nuclear Magnetic Resonance (NMR) Spectroscopy, and Synchronous Fluorescence Spectroscopy (Garcia, Martins, & Cabrita, 2013). (Giacalone, Giuliano, Gulotta, Monfreda, & Presti, 2015) reported how extra virgin olive oils of Italian and non-Italian origin were differentiated by GC-FID analysis of sterols and esterified sterols followed by chemometric tools. (Garrido-Delgado, del Mar Dobao-Prieto, Arce, & Valcárcel, 2015) described how combining a capillary column with Ion Mobility Spectrometry (IMS) enabled the quantification of 26 volatile metabolites (aldehydes, ketones, alcohols and esters) which might be used to verify the category of an olive oil sample. Garcia et al. (2013) applied

headspace solid-phase micro-extraction (HS-SPME) to quantify a selection of C6 compounds and terpene hydrocarbons as suitable markers of the geographical origin and genotype of the EVOO.

Today, significant research attention is also given to the development of analytical techniques which can be used to detect olive oil adulteration. Due to its economic importance olive oils are prone to such adulteration. Garcia et al. (2013) reviewed that blending of extra virgin olive oil (EVOO) with refined olive oil (ROO) is a common type of adulteration. Extra-virgin olive oil (EVOO) is also often adulterated with less expensive oils (e.g. sunflower, soy, corn, and rapeseed oils, hazelnut and peanut oils). Several analytical chemical techniques have been developed for the detection of olive oil adulteration such as liquid chromatography (analyzing triacylglycerol content), gas chromatography-mass spectrometry, mid-infrared spectroscopy, microwave reflectometry analysis, electronic nose, DNA identification of adulterant markers, detection of metabolite-based markers. (Borràs et al., 2015) studied mid-infrared (MIR) spectra (4000–600 cm^{-1}) of olive oils using chemometric methods to distinguish extra-virgin olive oils with lower quality olive oils. (Dais & Hatzakis, 2013) extensively reviewed current application of Nuclear Magnetic Resonance (NMR) Spectroscopy for olive oil quality and authenticity assessment. In particular two methodological approaches of metabolomics, metabolic profiling and metabolic fingerprinting were discussed in great detail.

Since the abovementioned techniques are rather time-consuming, often generate chemical taste, and require highly trained professionals, ongoing research attention is given in order to develop improved methods for vegetable oil adulteration. Using mass spectral characteristics of selected ions and equivalent chain length a model could detect adulteration of edible oil with other vegetable oils from a content of 10%. (Zhang et al., 2016) proposed ion mobility spectrometry (IMS) fingerprinting as a simple and rapid detection technology for adulteration.

Such IMS technology is based on the detection of ionized molecules that are separated under a weak electric field at ambient conditions. This innovative method proved to be able to classify sesame oil samples that were 10% adulterated. (Mabood et al., 2015) investigated the effect of thermal treatment (8 hours at 75°C) on the discrimination of pure extra virgin olive oil (EVOO) samples from EVOO samples adulterated with sunflower oil. Using fluorescence spectra adulterated oil samples could be identified from 2%. (van Wetten, van Herwaarden, Splinter, Boerrigter-Eenling, & van Ruth, 2015) investigated the applicability of fast DSC for the detection of sunflower oil (SFO) in EVOO. Heating curves of adulterated EVOOs showed an decrease in the one of two endothermic peaks which therefore could be used in the detection of adulteration of EVOO by SFO. Depending on the type of olive oil, the presence of 2–10% SFO

could be detected.

Based on the above literature overview it can be noticed that despite the recent advances in analytical methods for olive oil adulteration, minimal detection levels for adulteration are still too high. Additionally, techniques described above are typically time-consuming, require the use of harmful solvents and require highly expensive and complex technology and expertise. From literature it can be concluded that the currently available techniques are able to detect adulteration only from 2% oil addition, Garcia et al. (2013) concluded that further research is required to find new approaches or identify new compounds that could be assigned as reliable adulteration markers able to detect this fraudulent practice with high selectivity, sensitivity and accuracy.

In this work, the application of non-thermal plasma has been tested for the first time as an innovative preparation technique enabling the detection of low amounts of adulteration. In the recent study of Vandamme et al. (2015) and Van Durme, Nikiforov, Vandamme, Leys, & De Winne (2014) Non-Thermal Plasma (NTP) proved to be an accelerated oxidation technique with great potential to study and predict lipid oxidation phenomena and/or oxidative stability. It was proven that such non-thermal plasma treatments enabled a controlled and standardized accelerated induction of lipid oxidation in complex food matrices due to the generation of high concentrations of reactive species such as singlet oxygen, hydroxyl radicals, atomic oxygen, etc.. The application of non-thermal plasma technology to detect adulteration is unexplored, but highly promising since it can be assumed that each oil matrix oxidizes differently depending on its unique fatty acid profile, presence of polyphenols and specific composition of antioxidative compounds, etc. It is assumed that adulteration with different types of oil will result in small changes of oil composition and/or contents of minor compounds determining unique oxidation mechanisms and pathways. The result of such oxidative deterioration is the formation of volatile organic compounds. A forced non-thermal oxidation could thus result in the formation of an unique headspace composition that can be used to classify authentic from adulterated oil matrices. The goal of this work is therefore to investigate whether the application of non-thermal plasma might indeed induce such different lipid oxidation kinetics in adulterated olive oil samples, leading to the formation of a unique headspace composition.

EXAMPLES

Example 1 Non-Thermal Plasma treatment with the apparatus of present invention.

This example illustrates the formation after treatment by the invention of interesting unique volatiles that can be used to assess the treated product (in this case fish oil enriched with antioxidants)

5 Fish oil samples, either fresh or containing α -tocopherol at 1000 $\mu\text{g/g}$, based on Horn et al. (2009) were both treated with the plasma jet over a period of maximum 60 minutes. The temperature of each sample was measured directly after treatment, using a calibrated infrared thermometer (Votcraft, IR900-30S). Several temperature measurements of the sample during the plasma treatment revealed that no increase in temperature occurred. As described earlier NTP experiments were performed at a constant voltage input of 6.00 kV, while maintaining an
10 argon gas flow rate of 2 slm (standard liters per minute). The same analytical approach, using HS-SPME-GCMS, as described in Materials and Methods was used to evaluate the performance of the NTP. Moreover, addition of α -tocopherol should indicate if this new technique accelerates the lipid oxidation, with a more realistic prediction of the antioxidant properties of α -tocopherol.

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Following NTP-treatment, a significant increase of several lipid oxidation products was detected which were also found in the naturally aged fish oil. 2-propenal, 1-penten-3-one, pentanal, 2-undecanone, (E)-2-pentenal, (E)-3-hexenal, nonanal, hexanoic acid, butanoic acid and heptanal were the compounds that increased in concentration following the NTP-treatment.
20 These oxidation products are formed as a result of the reactive species present in the plasma jet, in particular atomic oxygen and singlet oxygen.

The compounds that increased in function of treatment time are displayed in figure 2c. Contrary, for a number of volatile lipid oxidation markers (e.g. (E,E)-2,4-heptadienal, (E)-2-decenal and 1-octen-3-ol) no significant increase was observed after NTP-exposure. This result could be
25 explained by the fact that the plasma jet was sustained by an argon gasflow of 2 slm, which creates a very turbulent atmosphere near the contact zone, causing a partial stripping of volatile compounds. Diffusion of volatiles from the oil matrix to the headspace is a well-known physical phenomenon which depends on various parameters, e.g. specific VOC/oil partitioning coefficient, temperature, turbulence... When the stripping effect is more dominant than the
30 formation of specific oxidation products, a decrease in concentration is observed. This might result in an underestimation of the formation rate of some volatile oxidation markers. Since the scope of this study is to evaluate to what extent the NTP treated sample correlates with a naturally aged sample, no further measurements have been performed on the stripped volatiles.

The addition of oxygen in the plasma results in the formation of several active species of which atomic oxygen and singlet oxygen are considered the most reactive. Singlet oxygen is an excited state of triplet oxygen (ground state) and highly reactive. This highly reactive oxygen species (ROS) can be formed in nature under influence of UV-light, and is responsible for photo-oxidation of lipids. Singlet oxygen directly reacts with an unsaturated fatty acid, without the prior formation of a radical, as is the case in the reaction mechanism with triplet oxygen. As discussed earlier, during the initiation step hydroperoxides are formed on the carbon atoms adjacent to a double bond, which in its turn leads to the formation of various secondary oxidation compounds through a various range of reaction mechanisms (Frankel, 1991). Pentanal can be formed from a 13-hydroperoxide of linoleic acid and the β -scission mechanism. (E)-2-Propanal in its turn can be formed from a 3-hydroperoxide of any omega-3 fatty acid including linoleic acid, DHA and EPA. Atomic oxygen is also a short-lived highly reactive species which also initiates the lipid oxidation mechanism by immediate reaction with the fatty acid, eventually leading to the formation of secondary oxidation products.

Based on the NTP oxidation experiment it could be concluded that the addition of α -tocopherol resulted in an antioxidant effect when added at 1000 $\mu\text{g/g}$ for most LOMs (in some cases no significant difference was observed). An additional NTP-treatment was performed on fish oil, enriched with 100 $\mu\text{g/g}$ α -tocopherol. In agreement with literature data, the antioxidative properties after adding 1000 $\mu\text{g/g}$ α -tocopherol were not observed when the same compound was added at 100 $\mu\text{g/g}$ concentration. This effect is clearly visible in case of pentanal, 2-undecanone, (E)-3-hexenal and nonanal. In case of 2-propenal and 1-penten-3-one, an antioxidative effect was observed. Heptanal on the other hand was formed much more rapidly when 100 $\mu\text{g/g}$ α -tocopherol was added, while an addition of 1000 $\mu\text{g/g}$ did not have a significant effect. Similar conclusions were made by Horn et al. (2009) who added different concentrations of γ -tocopherol to fish oil in order to evaluate the antioxidant effect. Addition of γ -tocopherol at concentrations above 440 $\mu\text{g/g}$ fish oil proved to result in a clear antioxidant effect, while addition at concentrations below 220 $\mu\text{g/g}$ resulted in a prooxidative effect. Prooxidative effects have been shown to rely on the ability of tocopherols to participate in side reactions in some food systems. It has been described that α -tocopherol reacts not only with peroxy radicals ($\text{ROO}\cdot$), but also with alkoxyl radical intermediates ($\text{RO}\cdot$) to form hydroxy compounds. Such side reactions may, to some extent, explain the present findings (Horn, Nielsen, & Jacobsen, 2009). Another explanation could be interactions between α -tocopherol and plasma-immanent species leading to the formation of oxidative compounds which in turn

lead to the pro-oxidative effect. Future research is needed to unravel these mechanisms. Based on these results, it can be concluded that the NTP-technique approaches the natural oxidation process more closely than the thermal oxidation test, considering the effects of α -tocopherol addition at different concentrations.

5

Example 2 Oil samples

The apparatus of present invention has been tested for verifying adulteration of olive oils (example), and any other matrix that can be oxidized.

Adulterated olive oil samples were prepared adding 1.00g, 2.00g and 3.00g from the same batch of sunflower oil (commercial available 100% refined sunflower oil) to respectively 99.0g, 98.0g and 97.0g from a batch of extra virgin olive oil (commercially available Carapelli Extra Virgin Olive Oil 'Classico', Firenze (Italy), immediately followed by a 10 minute intensive mixing. All samples were stored in the dark at -80 °C to prevent oxidation. The commercial sunflower oil had following composition; total saturated fat (11%), total mono-saturated fat (27.3%), total poly-unsaturated fat (61.7%). The commercial extra virgin olive oil (EVOO) was composed as; total saturated fat (14.5 %) (palmitic acid: 13.0%, stearic acid: 1.5%), total mono-unsaturated fat (70.3 – 73.5%) (oleic acid: 70.0%, palmitoleic acid: 0.3-3.5%), total poly-unsaturated fat (15.5%) (linoleic acid: 15.0%, α -linolenic acid: 0.5%).

Prior to analysis 0.5g of oil sample was carefully weighed in a 20 mL glass vial and sealed airtight with a silicon cap. Concentration of identified oxidation products were expressed semi-quantitatively, using an internal standard, 3 μ L methylpyrazine (14.33 mg/mL). All samples were measured in quadruplicate (n=4).

A dielectric barrier discharge (DBD) plasma source was used for Non-Thermal Plasma (NTP) treatments of the oil samples. The plasma jet was operated with oxygen (Ar/O₂) gas mixture. The species generated in the active zone of the discharge located in between electrodes can be divided in (listed according to increasing reactivity): charged particles (electrons, positive and negative ions); neutral excited states of Ar (metastables, resonance states and electron excited states); UV and VUV photons (appearing due to excimer radiation, OH and NO bands emission); oxygenated species including O₃, O₂ singlet, and O. For NTP-treatments, 20 g of oil sample was put in the glass container and placed in the plasma setup. Based on the work of Vandamme et al. (2015) treatments were performed using Ar/O₂-plasma (0.1% O₂) in argon atmosphere, based on its high correlation with the oxidation mechanism of oil samples at room temperature. Voltage input was kept at 6.00 kV in order to maintain a stable plasma jet. Samples

were treated for 60 minutes which is significantly shorter compared to other vegetable oil pretreatment techniques which typically take several hours (e.g. Rancimat, Active Oxygen Method, Schaal Oven) to days (storage tests). Moreover, from the work of Vandamme et al. (2015) it can be seen that shorter plasma treatment times might be sufficient. However, since the goal of this manuscript is to investigate the applicability of NTP for olive oil adulteration, optimization of the NTP pretreatment time is not of concern at this point.

Example 3 Analytical method (illustrative)

Isolation of the volatiles originated from lipid oxidation, was performed with an autosampler (multi-PurposeSampler® or MPS®, Gerstel®, Mülheim an der Ruhr, Germany), equipped with a headspace-solid phase microextraction unit. Solid-phase microextraction combined with one dimensional gas chromatography-mass spectrometry has been applied for analysis of olive oil aroma compounds, as well for the evaluation of lipid oxidation products in oils (Van Durme et al., 2014; Vandamme et al., 2015)

Chemical-analytical measurements that were performed in this work were both MS-fingerprinting based as conventional GC-MS profiling. In both cases a fully automated sample preparation unit (multi-PurposeSampler® or MPS®, Gerstel®, Mülheim an der Rur, Germany), combined with a 6890/5973 GC-MS system (Agilent Technologies®, palo Alto, CA) was used. Helium was used as a carrier gas (1 mL/min). When performing MS-fingerprinting analyses the GC column was continuously held at 250°C in order to avoid chromatographic separation of the analytes. Injector and transfer lines were maintained at 250°C and 280°C, respectively. The total ion current (70 eV) was recorded in the mass range from 40-230 amu (scan mode) using a solvent delay of 2 min and a run time of 5 min. For GC-MS profiling, the ZB-WAXplus column (30 m length, 0.25 LD., 0.25 µm film thickness) was programmed: 40°C (5 min) to 160°C at 3°C/min, from 160°C to 220°C at 5°C/min, held 3 minutes. Identification of volatile organic compounds in the vegetable oil headspace was performed by comparison with the mass spectra of the Wiley® 275 library. Confirmation of identification was done by determination of retention indices on the polar column after injection of a selection of authentic standards. Both experimentally and literature retention indices of identified compounds are inserted in Table 1.

Exploratory data analysis of the chemical information was performed by means of principal component analysis (PCA) (Unscrambler 9.7, Camo, Oslo, Norway). Using the same software also soft independent modelling of class analogy (SIMCA) was performed to determine the degree of difference based on calculated interclass distances. All data were weighted with 1/SD

(standardization) and a leverage correction was used to validate the models.

On chemical-analytical data paired comparison tests (t-test) were performed using SPSS Statistics 21 software to evaluate observe if differences between treatments were significant. Significances for the differences were established at an alpha risk of 5%.

5 Example 11 Classification of authentic and adulterated oil samples using MS-fingerprinting

Profiling of the headspace composition is an often applied approach to objectively assess the quality of food products. However despite the low detection limits that can be achieved, the detection of adulteration in oil matrices cannot be achieved using state-of-the-art headspace analysis. In this work headspaces of untreated and NTP-treated oil samples were analyzed using
10 MS-fingerprinting followed by a multivariate data processing. The headspaces were sampled by Solid-Phase MicroExtraction (SPME) using a CAR-PDMS fiber. This analytical method is an efficient and scientific accepted method to investigate whether samples can be classified from each other based on differences in headspace composition (Vandamme et al., 2015; Van Durme et al., 2014). As could be expected, Figure 3a illustrates that authentic EVOO and up to
15 3% Sunflower Oil (SFO) adulterated EVOO samples cannot be classified from each other based on conventional MS-fingerprinting data.

Samples of pure olive oil, 99% EVOO/1% SFO, 98% EVOO/2% SFO and 97% EVOO/3% SFO were each exposed to an identical NTP treatment. Based on previous works it was chosen to apply an O₂-doped Ar-plasma (Vandamme et al., 2015; Van Durme et al., 2014). It was
20 proven that such plasma configuration generates high concentrations of both atomic as singlet oxygen which are both highly oxidative species. Exposure of adulterated oil samples to these reactive species is expected to induce specific oxidation pathways which are adequately different than those induced in authentic EVOO. In order to verify this assumption, an identical MS-fingerprinting approach as discussed earlier was done on oil samples after exposure to the
25 NTP (Figure 1b).

The 2D-PCA-plot for NTP treated oil samples explains 97.53% (PC1: 93.01%, PC2: 4.52%) of the total variance. From Figure 3b it can be clearly seen that the 2D-PCA-plot shows well
30 clustered replicates for each olive oil sample illustrating the reproducibility of the HS-SPME-MS-fingerprinting method. Importantly, it can be seen that a NTP pretreatment on the olive oil samples confirmed the formation of an unique headspace composition that can be used to differentiate samples. Interestingly, adulterated olive oil samples score positive on the first principal component, while pure EVOO scored negatively. Additionally, a trend can also be

seen in relation to the degree of adulteration. Increasingly positive values for the second principal component are measured with increasing degree of SFO adulteration.

To confirm separate classification of NTP-pretreated adulterated olive oil samples, a statistical evaluation of the mass fingerprints by means of Soft Independent Modelling of Class Analogy (SIMCA) has been done (Table 3a and 3b). It is generally accepted that samples are significantly differentiated when interclass distances (IDs) are higher than 4.

Table 3a confirms the fact that no significant classification could be found between the untreated oil samples, since interclass distances were lower than 4 at all times. From Table 3b, it can be concluded that interclass distances between the extra virgin olive oil and the other samples, submitted to the NTP treatment, are mostly higher than 4. Interclass distances between 100% pure EVOO are in all cases higher than 19.5, confirming that even a 1% SFO addition results in significant changes of headspace composition enabling to detect a deviation from the pure olive oil. To the best of our knowledge, a detection limit of 1% SFO adulteration has never been achieved using headspace techniques. Next, it is clear from the relatively large interclass distances, that a NTP pretreatment will enable to detect SFO adulteration at even lower levels.

Headspace profiling by HS-SPME-GC-MS: evaluation of differences on authentic and adulterated olive oil before and after NTP pretreatment

Van Durme, Nikiforov, Vandamme, Leys, & Winne (2014) described how Non-Thermal Plasma exposure of lipids results in the formation of primary and secondary lipid oxidation products. Especially volatile secondary oxidation products proved to be the most useful markers for the evaluation of lipid oxidation (aldehydes, ketones, etc.). Table 4 represent chemical-analytical information of the headspace composition of both the authentic olive oil and the 1% SFO/99% EVOO blend. VOC profiling was performed on both matrices before and after NTP treatment. Each oil blend was treated during one single NTP treatment, previous work (Durme et al., 2014; Vandamme et al., 2015) sufficiently proved that NTP treatments are highly reproducible and stable. All measurements were done 4 times and average VOC concentrations were calculated using methylpyrazine as an internal standard.

From Table 4 it can be seen that the untreated 100% EVOO and 99% EVOO/1% SFO are highly similar. In all cases (except for 3-hexen-1-ol) concentrations of typically olive oil markers are not significantly different (t-test, $p < 0.05$). This confirms the abovementioned results, stating that HS-SPME-GC-MS profiling on untreated samples is not adequately

sensitive to distinguish authentic from adulterated olive oil.

As expected from MS-fingerprinting results, significant differences (t-test, $p < 0.05$) were measured for most volatiles after 100% EVOO and 99% EVOO samples were exposed to NTP. In particular the concentration of volatile secondary lipid oxidation products significantly increased after NTP treatment. Indeed, while the total concentration of aldehydes in untreated oil samples was average 11.558 $\mu\text{g/g}$, the NTP treatment resulted in the formation of increased aldehydes contents of respectively 18.939 $\mu\text{g/g}$ and 21.404 $\mu\text{g/g}$ for 100% EVOO and 99% EVOO. The same observations could be made for ketones and alcohols. A second observation is that NTP pretreatment resulted in amounts of secondary lipid oxidation markers that were significantly different between 100% EVOO NTP and 99% EVOO NTP. This was not the case without NTP exposure, hence this confirms that plasma exposure induces unique reactions depending on the purity of the oil sample.

Table 4 shows ratios between measured headspace concentrations after NTP treatment in both authentic and 99% EVOO. From this Table it can be seen that some marker molecules are produced in higher amounts in authentic olive oils (100% EVOO NTP), of which this observation was most pronounced for ethanol (ratio 7.708), 5-nonanol (ratio 4.450), 2-butanone (3.097), 2-pentyl furan (ratio 2.174) and (E)-2-octenal (ratio 1.588).

Contrary, other markers were present in significantly lower ($p < 0.05$) concentration in authentic compared to 1% SFO adulterated olive oil samples. This observation was most clear for nonanal (ratio 0.642), 6-methyl-5-hepten-2-one (0.708), (E)-4-undecenal (0.805) and decanal (0.816). Each of the aforementioned compounds are known oxidation markers. Morales et al. (2005) reviewed the occurrence and formation of off-flavors in olive oils due to for example oxidation processes. 2-octenal was one of the most odor active oxidation products in olive oils, equally saturated aldehydes such as nonanal were considered as main contributors to the rancid defect. The same effects, however to a lesser extent, is true for oxidation products nonanol and 6-methyl-5-hepten-2-one. 2-pentylfuran has been identified as useful marker which might be useful in distinguishing oxidation at late stages. Min & Boff (2002) reviewed that it is mainly singlet oxygen that is involved in the formation of 2-pentyl furan in vegetable oil ageing. It was reported that a hydroperoxide at carbon 10 of linoleic acid is required for the formation of this specific off-aroma compound. The presence of 2-pentyl furan is known to give a strong metallic off-flavor observed during sensory evaluations (Van Durme et al., 2014).

All vegetable oils consist of a complex mixture of saturated, mono-unsaturated and poly-unsaturated fatty acids, however in different ratios. Fatty acid composition is widely known as an important factor affecting the rate and outcome of lipid oxidation. Increasing number of unsaturated bonds in the lipid molecule lead to higher oxidative sensitivity. As sunflower oil generally contains higher amounts of poly-unsaturated fatty acids than virgin olive oil, adulteration of the latter with sunflower oil should logically result in formation of oxidation volatiles at a higher rate. This phenomena was observed for some oxidation markers such as nonanal (ratio 0.642), 6-methyl-5-hepten-2-one (0.708), (E)-4-undecenal (0.805) and decanal (0.816). However, many important lipid oxidation products such as 5-nonanol (ratio 4.450), 2-butanone (3.097), 2-pentyl furan (ratio 2.174) and (E)-2-octenal (ratio 1.588) were more present in pure oxidized olive oil. Although the general NTP-induced lipid oxidation phenomena occurring in vegetable oils were already discussed by Van Durme et al. (2014), at this stage of the research no concrete explanation can be given for the observed differences in headspace composition when olive oil is adulterated with sunflower oil. Since the lipid oxidation mechanism is known to have many important influencing parameters, such as oxygen concentration, water activity, presence of metal ions, free fatty acid content, presence of unique minor compounds such as polyphenols, antioxidants, prooxidants, etc., it would take a whole series of additional experiments to investigate the specific contribution of the aforementioned variables. More important is that results presented in this manuscript confirm that small changes in olive oil matrix composition result in significantly different NTP-induced oxidation chemistry. Based on the PCA results of NTP-treated oil samples, a good classification was achieved between the different oil samples, even below 1% adulteration. The aforementioned is only possible when NTP-induced headspace profiles are available for ascertained pure olive oils, so profiles of possibly adulterated batches can be compared. To the best of our knowledge no other available technique could manage to detect adulteration with such sensitivity. It can be concluded that non-thermal plasma proves to be an excellent preparative technique for the detection and quantification of adulteration of extra virgin olive oil with sunflower oil. Further research will indicate whether this method is applicable at even lower concentrations of adulteration, and other matrices.

Measurements of secondary oxidation volatiles during a natural storage test resulted in an accurate evaluation of the lipid oxidation process, thermal accelerated test and NTP-treatments. A natural aging test of 11 weeks resulted in the formation of many lipid oxidation volatiles, of which aldehydes proved to be the most important group. Compounds such as (E,E)-2,4-

Heptadienal, (E,Z)-2,6-Nonadienal, 1-octen-3-ol, (E)-2-decenal and others proved to be important oxidation compounds. Based on this natural oxidation test a list of lipid oxidation markers was chosen. Addition of 1000 µg/g α-tocopherol clearly resulted in an antioxidative effect in accordance with results found in another study of Horn et al. (2009).

- 5 The thermal accelerated lipid oxidation test, based on the well-known Rancimat test, proved to be insufficiently correlated with the natural aging of fish oil. Next to the formation of deviating types and concentrations of products and a different ratio of molecular groups, also a prooxidative effect was observed when 1000 µg/g of α-tocopherol was added.

The NTP-treatment resulted in the formation of several lipid oxidation products, which were also all found in the naturally aged fish oil, such as 2-propenal, (E)-2-pentenal, heptanal and 1-penten-3-one. However, other lipid oxidation markers found in the naturally aged fish oil, such as (E,E)-2,4-heptadienal and (E,E)-2,4-decadienal, did not seem to be formed during the NTP-treatment. This result could be explained by the highly turbulent atmosphere near the reaction zone, causing many volatiles to be stripped from the oil sample. In this way, an underestimation is made about the formation of oxidation products. Secondly, the addition of 1000 µg/g α-tocopherol resulted in a clear antioxidative effect, in accordance with the natural aging test. When 100 µg/g α-tocopherol was added however, prooxidative properties were correctly predicted. Non-thermal plasma proved to be able to accelerate the oxidation process in the fish oil, with a more accurate prediction of the antioxidative properties of α-tocopherol. In this way, the use of NTP as a non-thermal accelerated oxidation technique has more potential to evaluate additions of antioxidants than the thermal accelerated oxidation test.

The results from this work have provided some interesting insights into the use of NTP for accelerated lipid oxidation in fish oil. However, further research is required on this highly innovative and challenging plasma-technique. One important advantage of this plasma-technique is the high steerability. Many parameters, such as voltage, treatment time, oxygen concentration, configuration, water concentration and carrier gas can be altered, resulting in other plasma characteristics. When water is doped in the argon jet for example, a high concentration of hydroxyl radicals can be expected in the plasma. Since these highly reactive species are also responsible for natural oxidation processes, it could move the oxidation chemistry closer to natural oxidation. An important bottleneck of the used NTP configuration technique is the stripping of volatiles during the treatment. This could be overcome by treating the oil in a reaction chamber, and for example capturing the stripped volatiles in a solvent or sorbent tube, or measuring their concentrations by means of an electronic nose. Further experiments with this promising Non-Thermal Plasma for accelerated lipid oxidation in

complex food matrices will determine to what extent it can correlate to the natural aging process.

Particular and preferred aspects of the invention are set out in the accompanying independent and dependent claims. Features from the dependent claims may be combined with features of the independent claims and with features of other dependent claims as appropriate and not merely as explicitly set out in the claims.

Thus, the claims following the detailed description are hereby expressly incorporated into this detailed description, with each claim standing on its own as a separate embodiment of this invention.

Drawing Description

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will become more fully understood from the detailed description given herein below and the accompanying drawings which are given by way of illustration only, and thus are not limitative of the present invention, and wherein:

FIG. 1 is a schematic view showing the apparatus and its components such as the argon container (1), tungsten rod (2), quartz capillary (3), plasma jet (4), ground electrode (5), sintered glass disk (6), vacuum pump (7), peristaltic pump (8), chemical nose or VOC analyzer (9), software processor unit (10), outlet (11), power source (12), liquid sample (13) and grounding (14).

FIG. 1.a is a schematic view showing a non-thermal plasma (NTP) reactor with its integrated components such as the tungsten rod (2), ground electrode (5), plasma jet (4), inert layer of porous material (e.g. sintered glass disk) or a mesh (6) and interacting with a solid sample (13).

FIG. 1.b: NTP-treatment of fish oil: a) overall NTP-configuration; b) NTP-treatment of oil sample (detail); c) NTP-contact with fish oil sample

FIG. 2.a Evaluation of average headspace concentrations of typical fish oil oxidation products during 11 weeks of natural aging with and without addition of α -tocopherol antioxidant (AO) (1000 $\mu\text{g/g}$): a) 2-propenal, b) E-2-pentenal, c) 2-decenal, d) 1-octen-3-ol and e) (E,E)-2,4-octadienal (n=3).

FIG. 2.b. Evaluation of average headspace concentrations of typical fish oil oxidation products after 6 hours of thermal treatment with and without addition of α -tocopherol

antioxidant (AO) (1000 $\mu\text{g/g}$): a) 2-propenal, b) E-2-pentenal, c) 2-decenal, d) 1-octen-3-ol and e) (E,E)-2,4-octadienal (n=3).

FIG. 2.c. Average headspace concentrations of fish oil oxidation products after 60 minutes of NTP treatment with additional results of 100 $\mu\text{g/g}$ and 1000 $\mu\text{g/g}$ α -tocopherol antioxidant (AO) addition: a) propanal, b) propenal, c) pentanal, d) 2-undecanone, e) 3-hexenal, f) E-2-pentenal, g) nonanal, h) heptanal, i) 1-penten-3-one (n=3).

FIG. 3. 2D-Principal component analysis (PCA) biplot of the HS-SPME-MS-fingerprint data (n = 5) on both authentic EVOO samples (100%) as EVOO samples increasingly adulterated with sunflower oil (99%, 98%, 97%) without sample pretreatment (a) and after sample treatment with NTP (b).

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20

Table 1:

Compounds	concw0 (µg/g)	stdev	concw11 (µg/g)	stdev	OAV (-)	K _{exp}	K _{lit}	Idm
Alcohols								
ethanol	0.00	0.00	8.67	0.25	0.09	440	440	B
1-penten-3-ol	49.76	3.36	123.9	6.24	310	685	665	A
2-penten-1-ol	34.40	2.53	24.01	1.03	60	757	746	B
1-octen-3-ol	8.45	0.70	33.28	1.27	33278	959	968	A
Aldehydes								
acetaldehyde	0.00	0.00	15.59	0.28	130	560	427	C
2-propenal	0.00	0.00	110.3	1.40		572		C
propanal	8.10	0.16	38.99	0.42	1054	574	506	C
butanal	14.12	0.99	9.93	0.45	268	601	596	B
2-butenal	49.96	1.06	92.59	1.51		637	623	B
pentanal	0.00	0.00	25.28	1.37	2106	677	690	A
2-butenal, 2-methyl-	0.00	0.00	43.19	3.04		696	611	A
E-2-pentenal	0.00	0.00	53.99	1.23	36	718	731	B
trans,trans-2,4-heptadienal	367.4	16.7	517.9	17.10	34527	1000	996	B
2-octenal	64.10	4.09	55.23	3.80	18411	1052	1056	B
E,E-2,4-octadienal	0.00	0.00	25.69	1.67		1088	1084	B
Z,Z-2,4-octadienal	0.00	0.00	41.08	1.75				C
nonanal	0.00	0.00	16.76	0.19	16757	1102	1083	A
2,6-nonadienal (E,Z)	0.00	0.00	24.99	1.13	2489223	1144	1154	B
2-Nonenal	0.00	0.00	51.74	1.65	517429	1152	1147	B
2,4,6-octatrienal	0.00	0.00	15.91	0.66		1161		C
2-decenal	0.00	0.00	31.95	3.03	106510	1246	1250	B
2,4-decadienal	0.00	0.00	17.87	2.22	255313	1273	1297	A
Aromatics								
benzene	34.30	1.26	4.05	0.21				C
3,4-dihydropyran	0.00	0.00	7.74	0.27		680	705	B
2-Ethylfuran	144.2	11.0	94.75	2.45		502	698	C
2-methylfuran	11.05	0.56	27.41	0.86		715	633	C
2-Methoxyfuran	0.00	0.00	33.03	0.63		810		C
phenol	10.84	1.36	10.98	0.11	1.9	974	981	B
2-(2-propenyl)-furan	0.00	0.00	24.12	0.67		1008		C

Carbon acids								
formic acid	0.30	0.08	243.4	3.73	0.54	510	543	B
acetic acid	25.59	11.7	242.8	7.41	2.4	572	600	B
ester								
Isopropyl dodecanoate	0.00	0.00	10.79	0.97				C
hydrocarbons								
2-pentene, 4-methyl-	0.00	0.00	4.28	0.11				C
2,5-octadiene	264.9	7.65	19.13	0.97		812		C
octatriene, 1,3-trans-5-trans-	39.61	4.43	43.37	2.36		878		C
2,4,6-octatriene	33.59	3.20	6.86	0.25		935		C
1-acetyl-1-cyclohexene	0.00	0.00	25.40	0.37		943		C
1-tridecene	7.83	1.22	9.00	0.68		1286	1289	B
tridecane	12.72	0.68	16.81	1.17		1299	1300	A
tetradecane	18.25	1.23	17.83	0.10		1419	1400	A
hexadecane, 2,6,10,14-tetramethyl	0.00	0.00	11.32	0.81				C
1-pentadecene	0.00	0.00	23.40	3.36		1538	1540	B
pentadecane	287.9	59.0	655.7	70.74		1538	1500	A
hexadecane	27.57	4.19	31.08	7.90		1600	1600	A
heptadec-8-ene	2.17	1.14	26.53	2.90				C
1-heptadecene	6.44	0.22	85.31	4.62				C
heptadecane	36.37	10.5	260.9	22.44		1674	1700	A
pentadecane, 2,6,10,14-tetramethyl-	0.00	0.00	71.48	5.04				C
isoprene	0.00	0.00	6.42	0.41				C
ketones								
ethyl vinyl ketone	26.66	1.77	43.13	2.31	43133			C
1-hydroxy-2-butanone	0.00	0.00	15.56	1.24	0.31	694	674	A
5-ethyl-2(5H)-furanone	0.00	0.00	152.9	11.12		925	954	B
ethanone, 1-(1-cyclohexen-1-yl)-	0.00	0.00	80.01	6.03		943		C
1-octen-3-one	0.00	0.00	14.40	0.00	2879850	972	1040	B
3,5-octadiene-2-one	25.33	2.19	85.68	4.82	71403	1063	1040	B
2-nonanone	23.27	0.90	18.53	0.79	93	1090	1093	B
2-undecanone	4.48	1.14	11.61	0.05	1659	1277	1296	B

Table 2:

Compound	Odor/aroma	OTV ($\mu\text{g/g}$)	11 w NA ($\mu\text{g/g}$)	6h 100°C ($\mu\text{g/g}$)	60 min O ₂ /Ar ($\mu\text{g/g}$)
1-penten-3-ol	mushroom	0.4	123.9	290.5	-
2-propenal	burnt cooking grease	-	110.3	168.5	11.9
propanal	solvent, pungent	0.0	39.0	45.5	5.7
E-2-pentenal	strawberry, fruit, tomato	1.5	54.0	1093.2	6.3
heptanal	soap, fat, almond	0.0	-	716.3	22.6
(E,E)-2,4-heptadienal	nut, fat	0.0	517.9	6520.0	-
2-nonenal	orris, fat, cucumber	0.0	51.7	667.8	-
2,6-nonadienal	cucumber, wax, green	0.0	25.0	334.1	-
2-decenal	tallow	0.0	32.0	130.9	-
3,5-octadien-2-one	fruity, green, grassy	0.0	85.7	186.0	-
1-penten-3-one	Earthy, Green, Pungent	1.3	43.1	320.4	5.9
3-hexenal	grassy, green	0.3	-	-	6.1
nonanal	floral, waxy, green	1.0	16.8	381.9	28.1
2-undecanone	fruity-rosy, orange-like	7.0	11.6	59.9	19.3
(E,E)-2,4-octadienal	fatty	-	25.7	220.0	-

- 5 OTV = OdorThreshold Value, (11 w NA) = 11 weeks of Natural Aging, (6h 100°C) = thermal oxidation test of 6 hours at 100 °C, (60 min O₂/Ar) = Oxygen/ Argon Non-Thermal Plasma treatment for 60 minutes.

Table 3a & 3b

a)

	100% EVOO	99% EVOO	98% EVOO	97% EVOO
100% EVOO	0.000	0.755	2.991	2.497
99% EVOO	0.755	0.000	2.627	2.190
98% EVOO	2.991	2.627	0.000	0.105
97% EVOO	2.497	2.190	0.105	0.000

5 b)

	100% EVOO_NTP	99% EVOO_NTP	98% EVOO_NTP	97% EVOO_NTP
100% EVOO_NTP	0.0	19.5	22.9	20.8
99% EVOO_NTP	19.5	0.0	5.7	8.9
98% EVOO_NTP	22.9	5.7	0.0	3.6
97% EVOO_NTP	20.8	8.9	3.6	0.0

Table 3: Interclass distances after SIMCA data processing on MS-fingerprinting results of Extra Vierge Olive Oils samples with increasing degree of sunflower oil adulteration. Table 3a represents interclass distances of untreated oil samples, while Table 3b those of NTP-pretreated oil samples

10

Table 4

Compound**	Kl _{exp}	Kl _{lit}	Untreated		NTP pretreated		RATIO 100%/99 %
			100% EVOO Cone (µg/g)	99% EVOO Cone (µg/g)	100% EVOO Cone (µg/g)	99% EVOO Cone (µg/g)	
Aldehydes							
pentanal ^B	983	978	0.177 ± 0.122	0.217 ± 0.012	0.305 ± 0.039	0.227 ± 0.008	1.342*
Hexanal ^A	1077	1077	0.608 ± 0.022	0.592 ± 0.016	1.949 ± 0.141	1.362 ± 0.009	1.431*
Heptanal ^B	1149	1174	0.127 ± 0.014	0.137 ± 0.009	1.700 ± 0.111	1.744 ± 0.051	0.975
(E)-2-hexenal ^B	1179	1196	5.819 ± 0.117	5.658 ± 0.095	1.862 ± 0.060	1.618 ± 0.028	1.150*
Octanal ^B	1275	1278	0.250 ± 0.017	0.257 ± 0.016	1.367 ± 0.053	1.584 ± 0.019	0.863*
(E)-2-heptenal ^B	1309	1334	2.984 ± 0.115	2.951 ± 0.064	1.134 ± 0.055	0.901 ± 0.045	1.258*
Nonanal ^A	1395	1395	1.123 ± 0.038	1.214 ± 0.105	5.296 ± 0.129	8.254 ± 0.305	0.642*
(E)-2-octenal ^B	1438	1416	0.246 ± 0.030	0.209 ± 0.030	1.012 ± 0.059	0.637 ± 0.035	1.588*
Decanal ^B	1529	1500	0.129 ± 0.010	0.136 ± 0.034	1.994 ± 0.110	2.445 ± 0.095	0.816*
2-Nonenal ^B	1563	1540	0.000 ± 0.000	0.000 ± 0.000	0.513 ± 0.028	0.504 ± 0.021	1.018
(E)-4-undecenal ^C	1569	/	0.000 ± 0.000	0.000 ± 0.000	0.272 ± 0.011	0.337 ± 0.033	0.805*
(E)-4-decenal ^C	1685	/	0.000 ± 0.000	0.000 ± 0.000	1.169 ± 0.095	1.343 ± 0.118	0.871*
(E)-2-dodecenal ^B	1799	1842	0.039 ± 0.026	0.064 ± 0.010	0.208 ± 0.022	0.275 ± 0.027	0.756*
(E,E)-2,4-decadienal ^B	1807	1844	0.086 ± 0.006	0.091 ± 0.011	0.158 ± 0.012	0.172 ± 0.017	0.918
Total aldehydes			11.588	11.528	18.939	21.404	0.885

Esters		Ketons		Alcohols	
2-methyl-2-propenoic acid, methyl ester ^B	993	1008	0.000	± 0.000	0.000
3-hexen-1-ol, acetate ^B	1368	1337	0.341	± 0.014	0.343
Total esters			0.341		0.343
2-butanone ^B	961	905	0.000	± 0.000	0.000
2-octanone ^B	1270	1255	0.111	± 0.006	0.111
1-octen-3-one ^B	1287	1299	0.000	± 0.000	0.000
6-methyl-5-hepten-2-one ^B	1328	1341	0.124	± 0.006	0.127
total ketones			0.234		0.239
Ethanol ^A	926	926	0.426	± 0.050	0.453
1-hexanol ^A	1371	1371	0.408	± 0.273	0.524
3-hexen-1-ol ^B	1388	1392	0.901	± 0.027	0.834
(E)-2-hexen-1-ol ^B	1417	1410	0.889	± 0.025	0.866
1-octanol ^B	1602	1555	0.202	± 0.020	0.192

1-nonanol ^B	1660	1666	0.000	± 0.000	0.000	± 0.000	0.169	± 0.016	0.172	± 0.008	0.982
2-decen-1-ol ^B	1714	1794	0.111	± 0.010	0.111	± 0.011	0.219	± 0.026	0.255	± 0.012	0.862*
<i>Total alcohols</i>			2.937		2.979		6.836		3.043		2.247
<u>Other</u>											
2-pentylfuran ^B	1204	1198	0.000	± 0.000	0.000	± 0.000	1.265	± 0.077	0.582	± 0.029	2.174*
methylpyrazine (I.S.) ^A	1264	1264	85.980	± 0.000	85.980	± 0.000	85.980	± 0.000	85.980	± 0.000	1.000*
Acetic acid ^A	1465	1465	2.045	± 1.371	2.116	± 0.792	3.994	± 0.148	0.000	± 0.000	
<i>Total others</i>			88.025		88.096		97.239		86.562		1.054
TOTAL VOC			118.226		118.273		148.961		140.154		1.063

5

Claims**What is claimed is:**

1. An apparatus to accelerate oxidation by non-thermal plasma in a sample comprising an organic product, the apparatus comprising a reaction chamber equipped with a build-in plasma reactor and a build in sample holder comprised in the reaction chamber, whereby the reaction chamber is adapted for airtight closure (by seal, an air lock) so that it can operate or under atmospheric conditions or under a selected gas atmospheres and whereby the sample holder is functionally connected or connectable with a volatile trap in such a way that a volatiles released from said the composition it is trapped.
2. The apparatus according to claim 1, whereby the reaction chamber is connected with gas conduit so that when operational gas loaded with volatile oxidation products is hyphenated to the volatile trap in such a way that volatiles released from a sample under non-thermal plasma (NTP) treatment is trapped.
3. The apparatus according to any one of the previous claims 1 to 2, whereby the volatile trap is a cooled trap.
4. The apparatus according to any one of the previous claims 1 to 3, whereby the trap is a cryo trap.
5. The apparatus according to any one of the previous claims 1 to 4, whereby the trap is a liquid nitrogen cooled cryo trap.
6. The apparatus according to any one of the previous claims 1 to 5, whereby the trap comprises a tube containing sorbent material, for instance tenax, silica gel or activated charcoal or it comprises an ice-cooled water sample for the volatile organic compounds to be guided or bubbled into.
7. The apparatus according to any one of the previous claims 1 to 6, whereby the cooled trap is connected or followed by a volatile organic compounds (VOC) analyser.
8. The apparatus according to any one of the previous claims 1 to 7, whereby the apparatus has at least one enclosure for connecting or removing the sample holder.
9. The apparatus according to any one of the previous claims 1 to 8, whereby the enclosure is adaptable or removable so that the apparatus can comprise at the position under the NTP different types of sample holders such as a fountain system holder, a solids/liquid sample holder, a cone samples holder, a cube sample holder, a cylindrical sample holder.
10. The apparatus according to any one of the previous claims 1 to 9, comprising an

- 5 enclosure to fix a mesh above sample holder such mesh being non-thermal plasma (NTP) penetrable.
11. The apparatus according to any one of the previous claims 1 to 10, with a sample holder that comprises an attachment means or an enclosure on top so that it can be covered by a non-thermal plasma (NTP) penetrable mesh.
- 10 12. The apparatus according to any one of the previous claims 1 to 11, whereby the VOC analyzer comprises a detectors of the group consisting of an electronic nose, flame ionization detector (FID), mass spectrometry (MS), ultraviolet (UV), infrared (IR), time-of-flight mass Spectrometer (TOF) and selective ion flow tube-mass spectrometry (SIFT-MS).
13. The apparatus according to any one of the previous claims 1 to 12, whereby the reaction
15 chamber is 1) connected or connectable to a gas fluid pump for extracting said fluid, such as air, from the reaction chamber and whereby the reaction chamber is 2) connected to at least one gas container for introduction of the specific gas, for example helium or argon.
14. The apparatus according to any one of the previous claims 1 to 12, whereby when
operational the gas pump and the gas container are for replacing ambient air in said the reaction
20 chamber by a gas of choice.
15. The apparatus according to any one of the previous claims 1 to 12, whereby the sample position of the sample holder is controllable such that when operational the sample position can be placed in or under the plasma discharge.
16. The apparatus according to any one of the previous claims 1 to 15, whereby the non-
25 thermal plasma reactor is installed inside the airtight reaction chamber of aid apparatus.
17. The apparatus according to any one of the previous claims 1 to 16, whereby the non-thermal plasma (NTP) reactor to generate oxidative reactive species on a non-thermal way is any one of the group consisting of a corona discharge reactor, dielectric barrier discharger (DBD), plasma jet discharger (one or two electrodes are covered with dielectric barrier material)
30 and glow discharger.
18. The apparatus according to any one of the previous claims 1 to 18, whereby the energy source for non-thermal plasma is dielectric barrier discharge or radio frequency discharge.
19. The apparatus according to any one of the claims 1 to 19, whereby the electrodes non-thermal plasma reactor have any one of the following shapes pin shaped, hollow pin, conic or
35 flat.
20. The apparatus according to claim 19, whereby the electrodes are at a distance of 0.1 mm to 1 mm in between.
21. The apparatus according to any one of the claims 1 to 20, whereby the materials of the

- 5 electrodes comprise Tungsten, stainless steel, copper, copper alloy.
22. The apparatus according to any one of the claims 1 to 21, whereby gas container is connected or connectable to the reaction chamber.
23. The apparatus according to any one of the claims 1 to 22, whereby the connection between the gas container and the reaction chamber comprises comprises a fluid controller to
10 control the gas flow.
24. The apparatus according to any one of the claims 1 to 23, whereby the gas container outlet or the reaction chamber inlet comprises a fluid controller to control the gas flow to sustain a constant plasma discharge can be varied between 0.001 sLm up to 5 sLm (sLm = standard litres per minute).
- 15 25. The apparatus according to any one of the claims 1 to 24, whereby the gas container when partially or totally filled comprises a gas of the group consisting of pure argon, pure helium, argon:air mixture, argon: helium mixture, Ar/O₂, He/O₂, Ar/H₂O, He/H₂O.
26. The apparatus according to any one of the previous claims 1 to 25, characterized in that said apparatus is operational in two modi operandi: a) as a stand-alone unit to pre-treat
20 introduced sample matrices or b) hyphenated with a VOC (Volatile Organic Compounds) analyser.
27. The apparatus according to any one of the previous claims 1 to 26, characterized in that the connection between the gas container and the reaction chamber is a high-pressure tubing.
28. The apparatus according to any one of the previous claims 1 to 27, whereby the
25 apparatus comprises a liquid pump to so that a liquid sample can be pumped through a sintered glass disk into the sample holder position under the non-thermal plasma (NTP).
29. The apparatus according to any one of the previous claims 1 to 28, whereby the liquid sample introduction comprises a sintered glass fountain.
30. The apparatus according to any one of the previous claims 1 to 29, whereby the liquid
30 samples holder can be position so that the NTP is close to the liquid sample or so that the NTP discharges inside the liquid sample.
31. The apparatus according to any one of the previous claims 1 to 30, whereby a solid sample holder can be position so that the NTP is close to the solid sample or so that the NTP discharges on the solid sample.
- 35 32. The apparatus according to any one of the previous claims 1 to 31, adapted for online evaluation of VOC formation by the reaction chamber connected with gas conduit so that when operational gas loaded with volatile oxidation products is hyphenated to a VOC analyzer on the gas conduit or at the outlet of gas conduit.

- 5 33. The apparatus according to any one of the previous claims 1 to 32, whereby the sample holder is connectable and removable by and enclosure in the reaction chamber under the non-thermal plasma reactor.
34. The apparatus according to any one of the previous claims 1 to 33, comprising enclosures to hold a plurality of sample holders functionally connected with a motor so that
10 separate sample holders can be moved under the NTP.
35. The apparatus according to any one of the previous claims 1 to 34, comprising at least one sampler holder with a volume in a range from 10 μ L to 10 000 mL.
36. The use of the apparatus according to any one of the previous claims 1 to 35, to generate accelerated ageing in said organic sample
- 15 37. The use of the apparatus according to any one of the previous claims 1 to 35 to generate accelerated oxidation chemistry in said organic sample

FIGURES

Figure 1

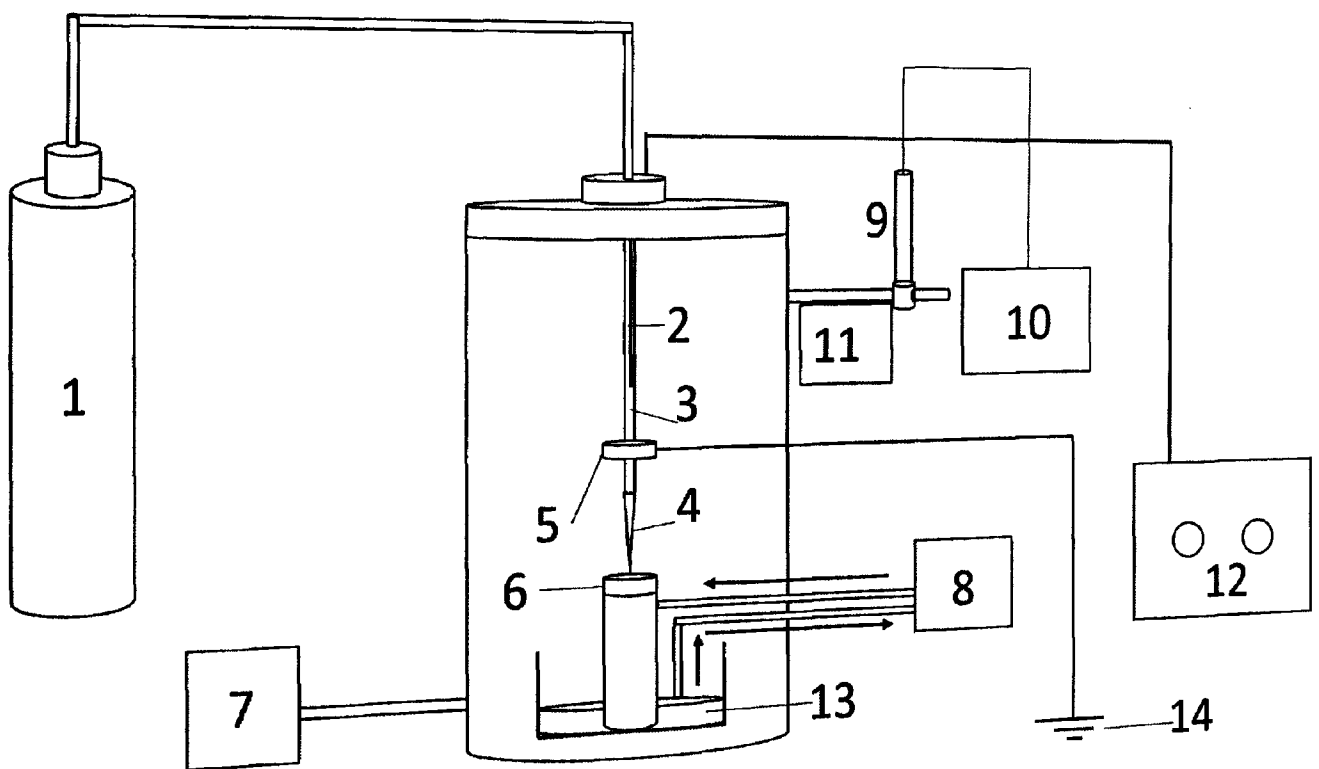


Figure 1a

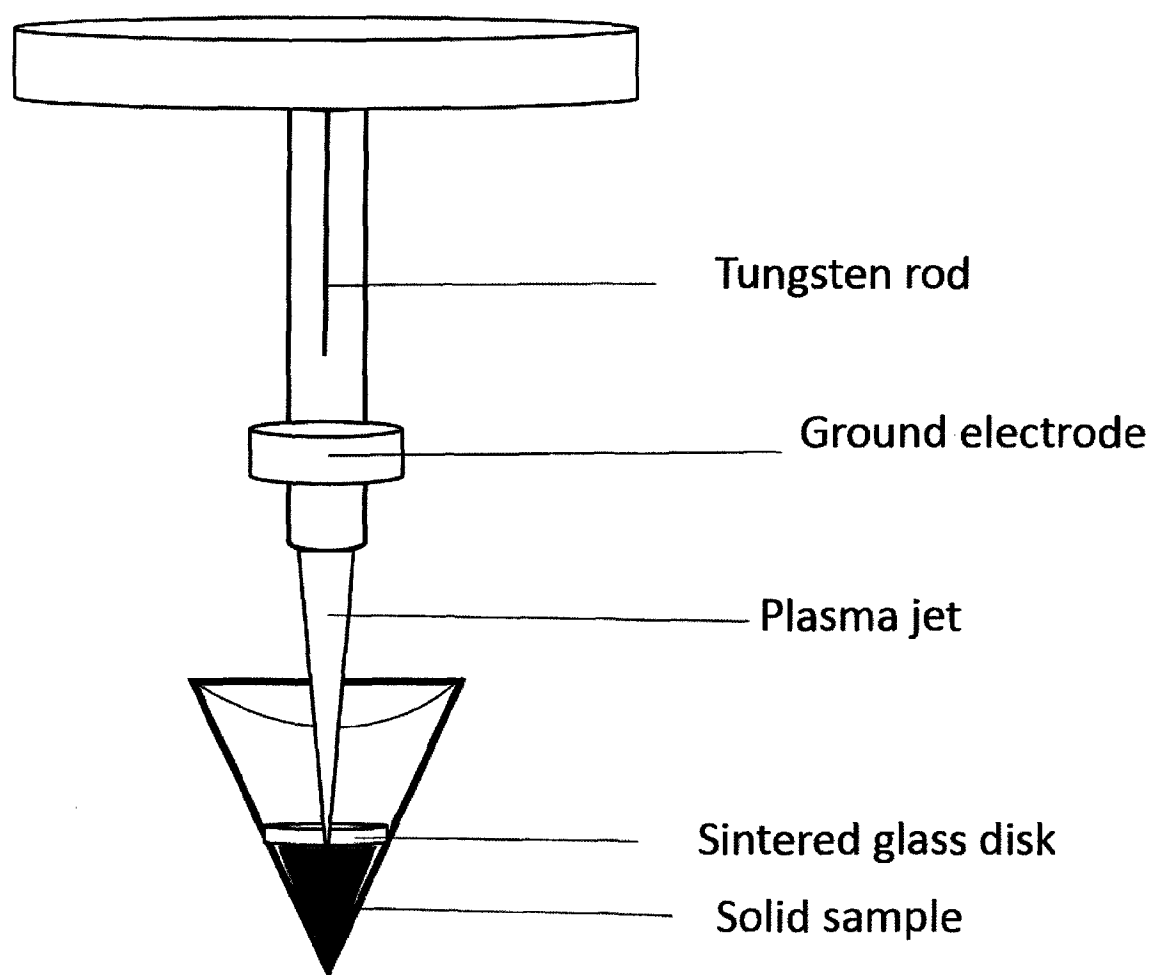


Figure 1b

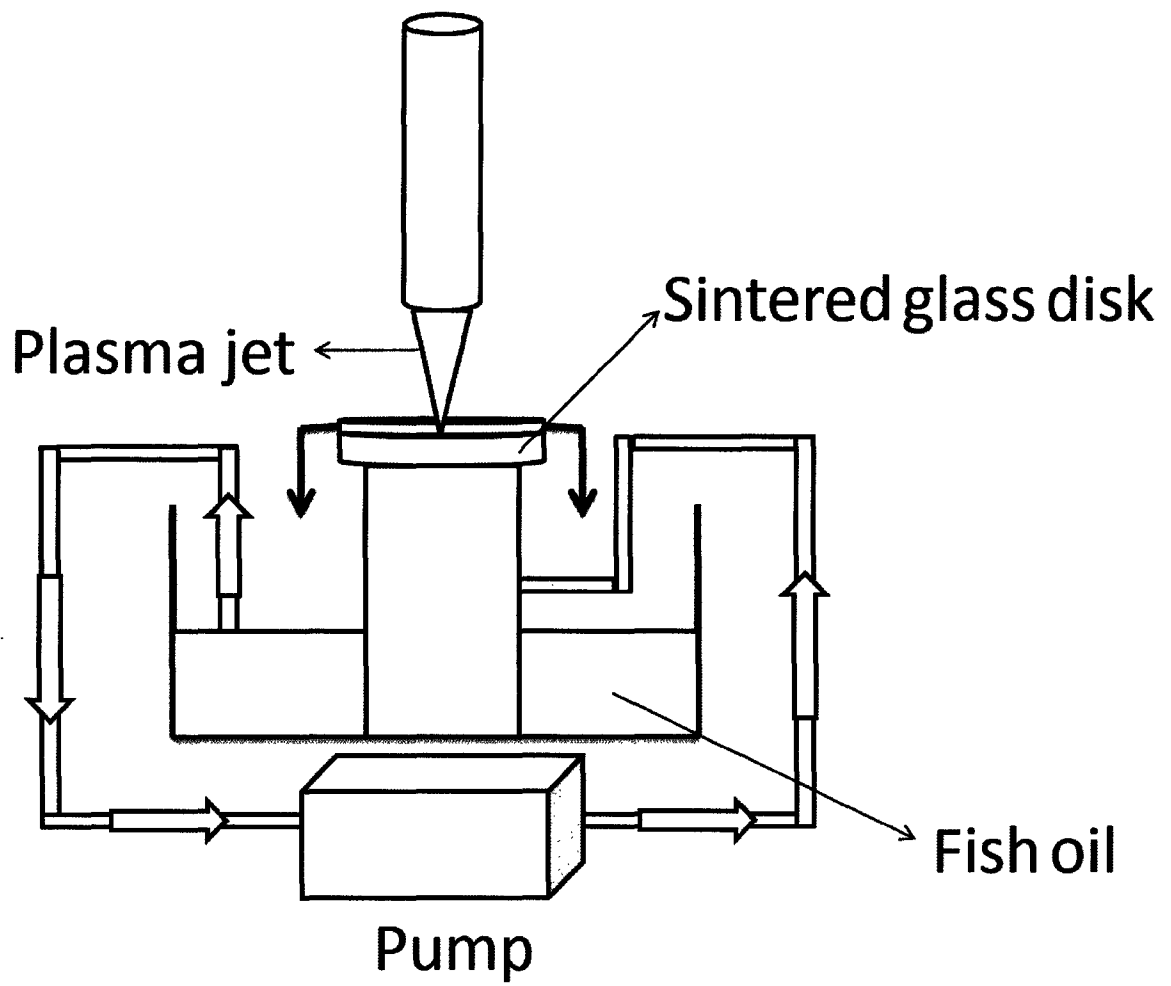
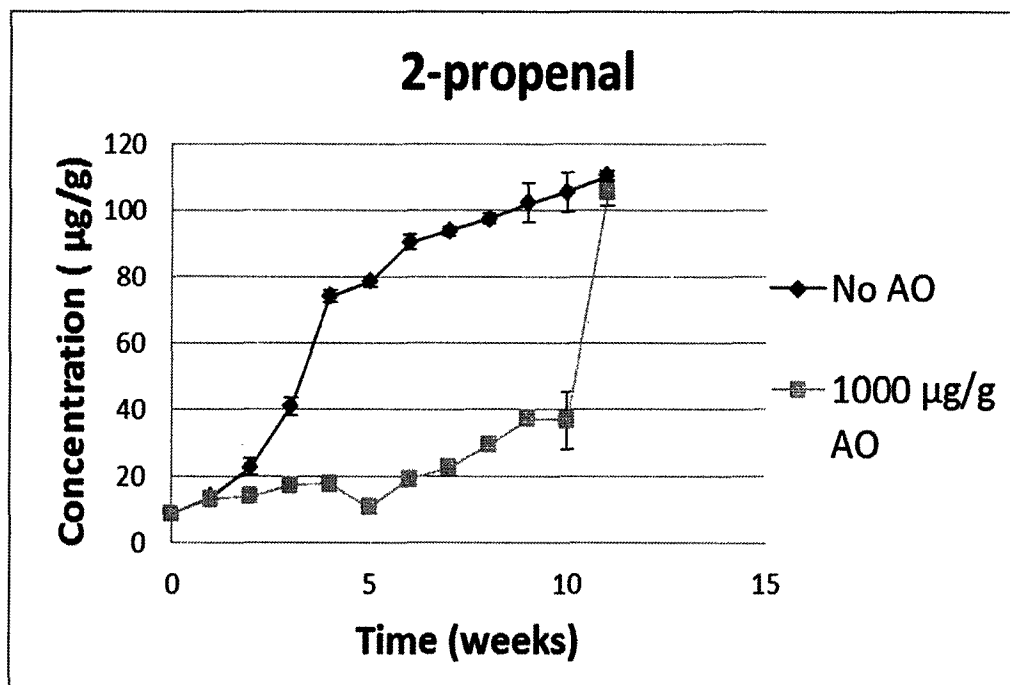


Figure 2a

a)



b)

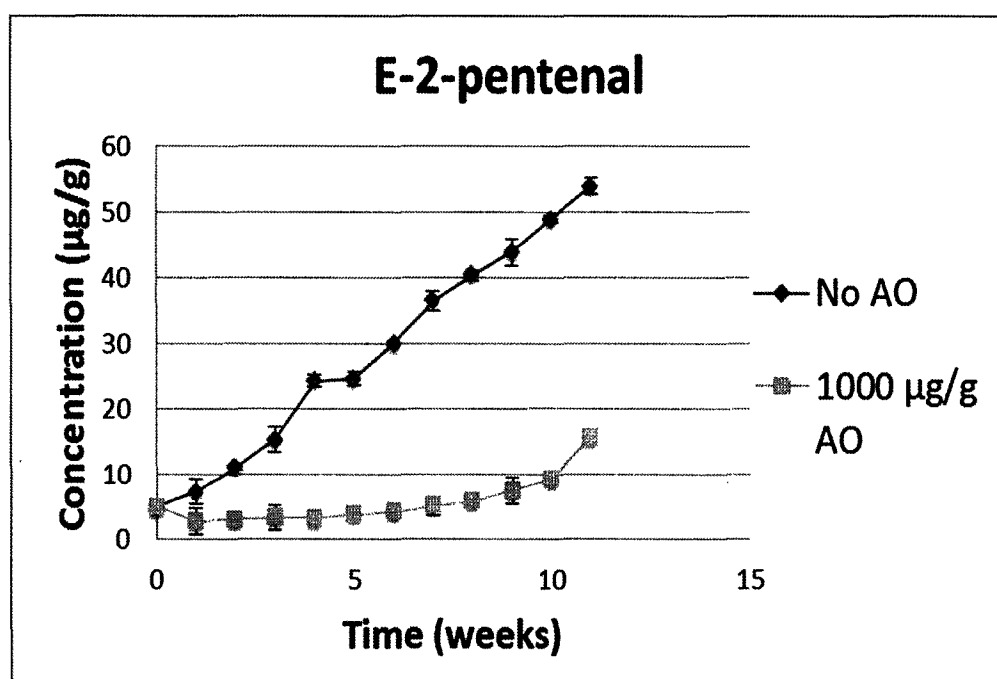
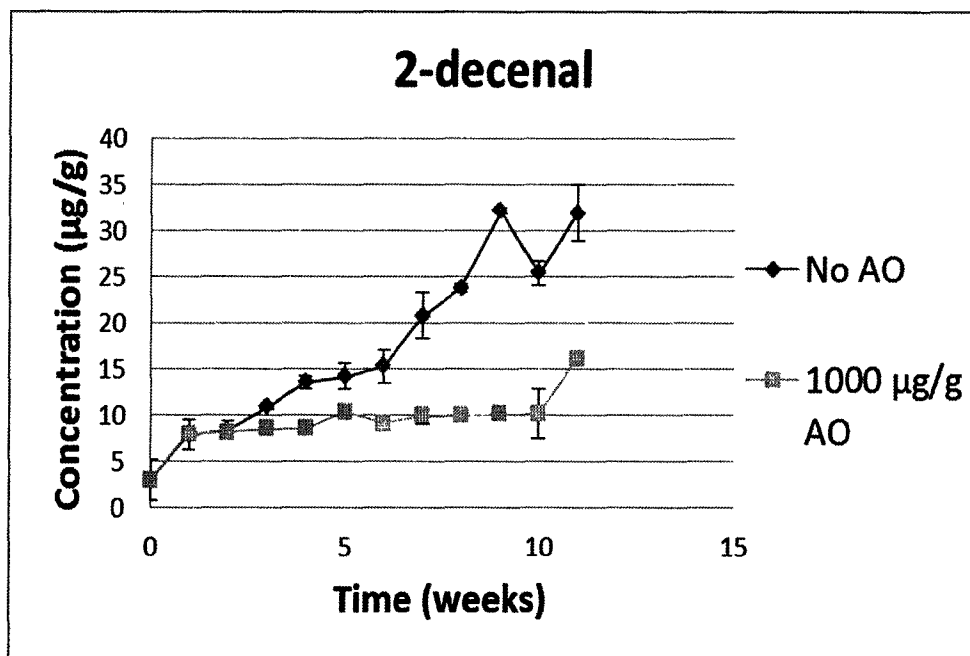


Figure 2a (continued)

c)



d)

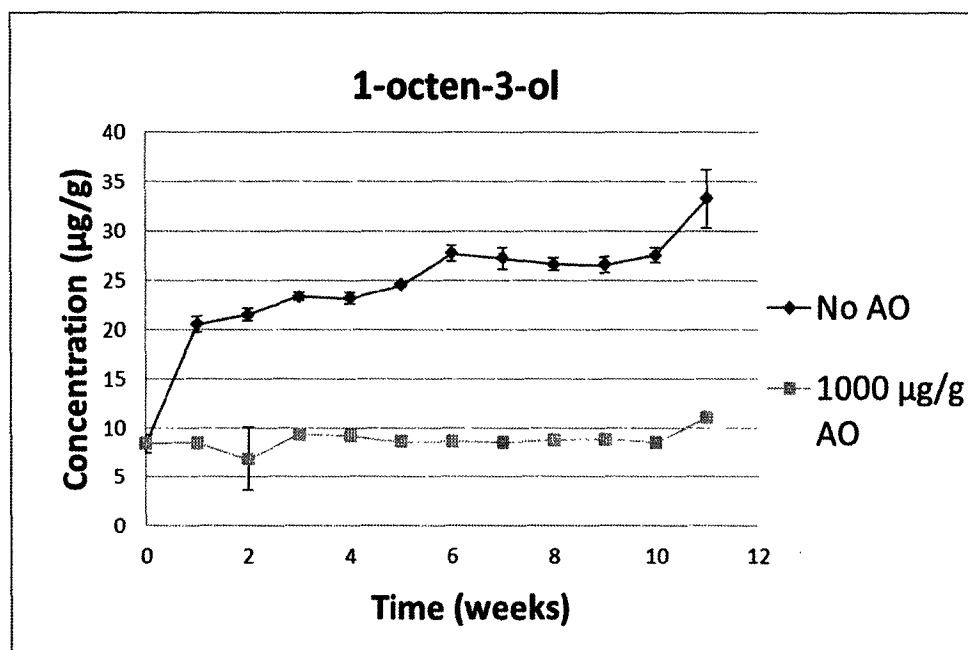


Figure 2a (continued)

e)

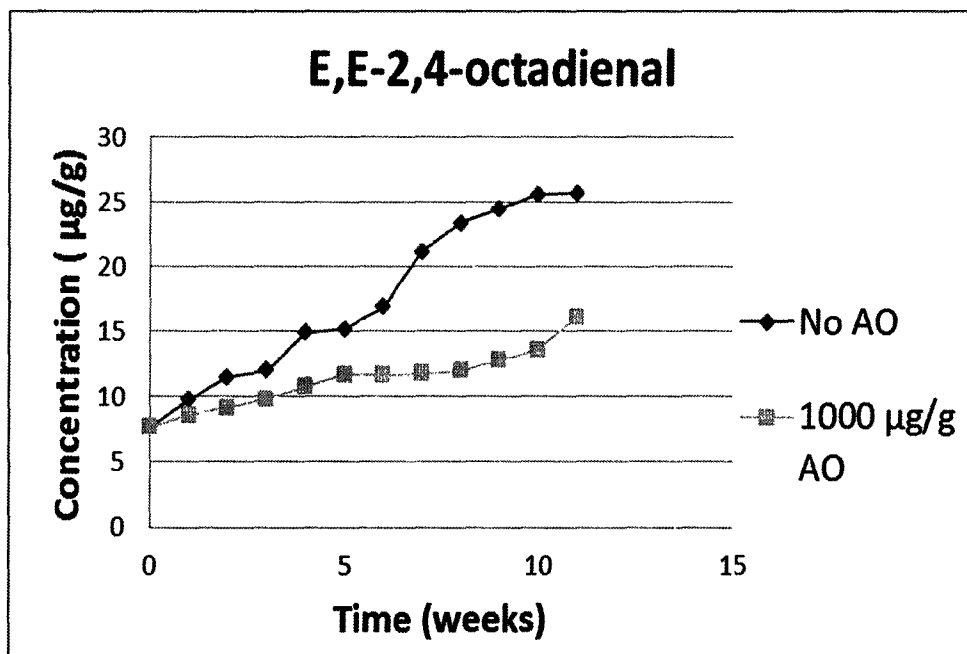
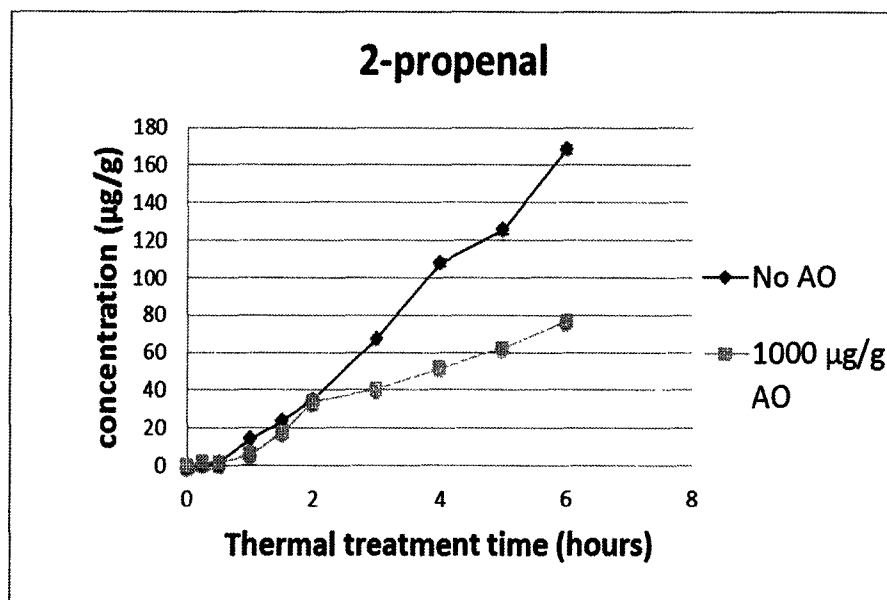


Figure 2b

a)



b)

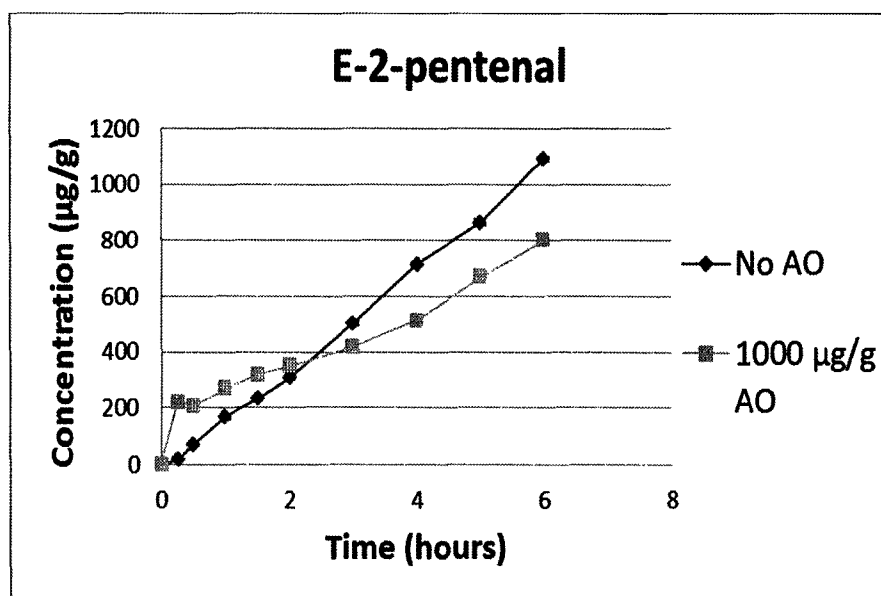
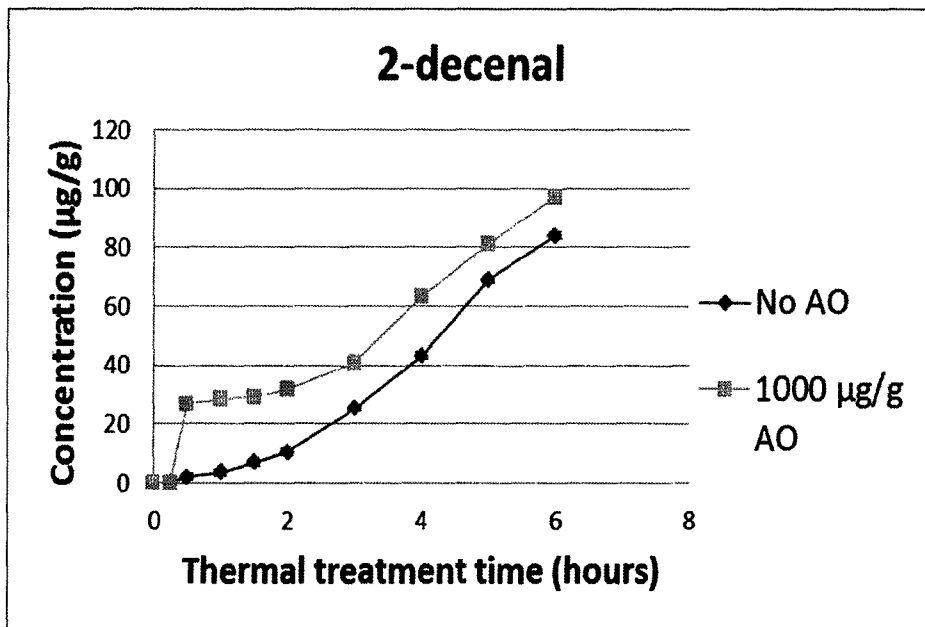


Figure 2b (continued)

c)



d)

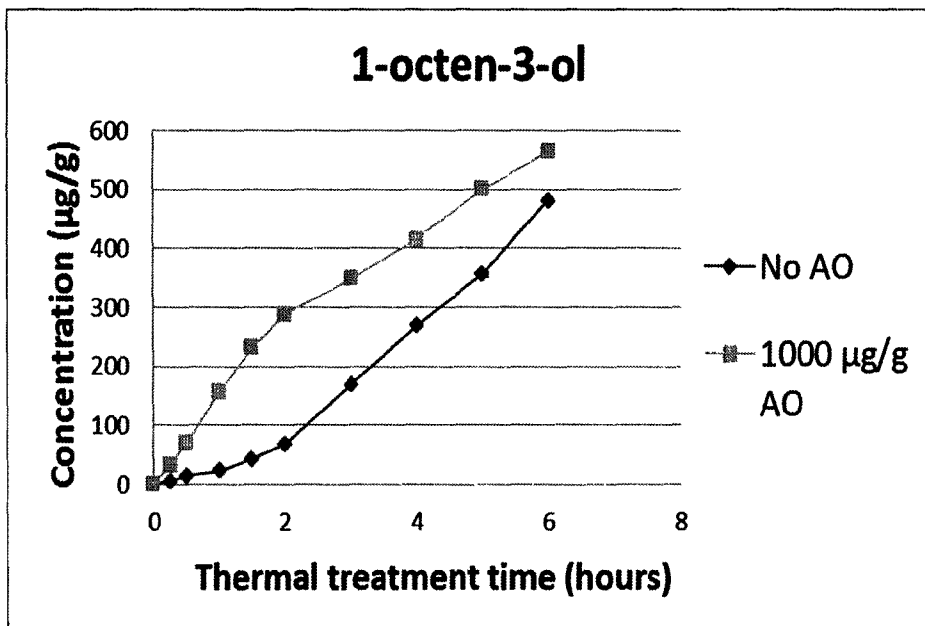


Figure 2b (continued)

e)

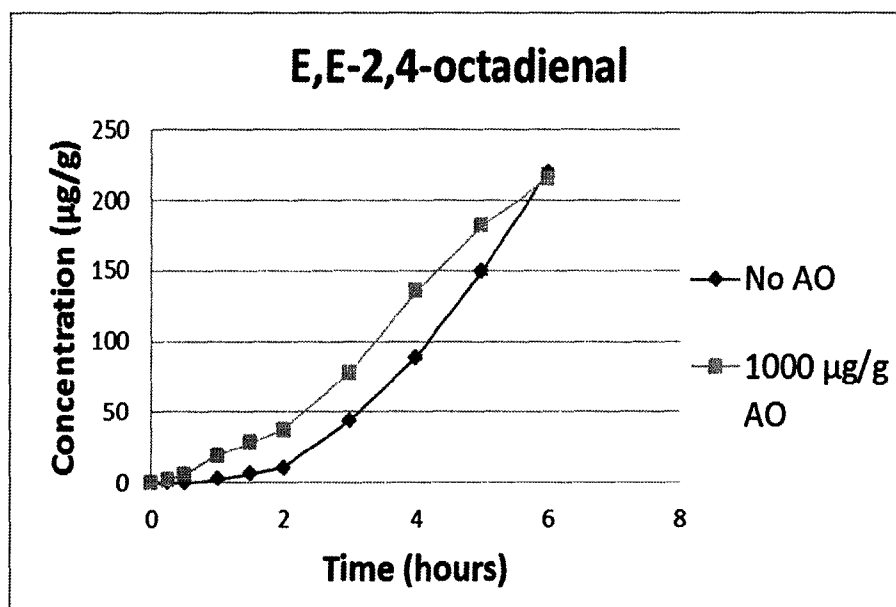
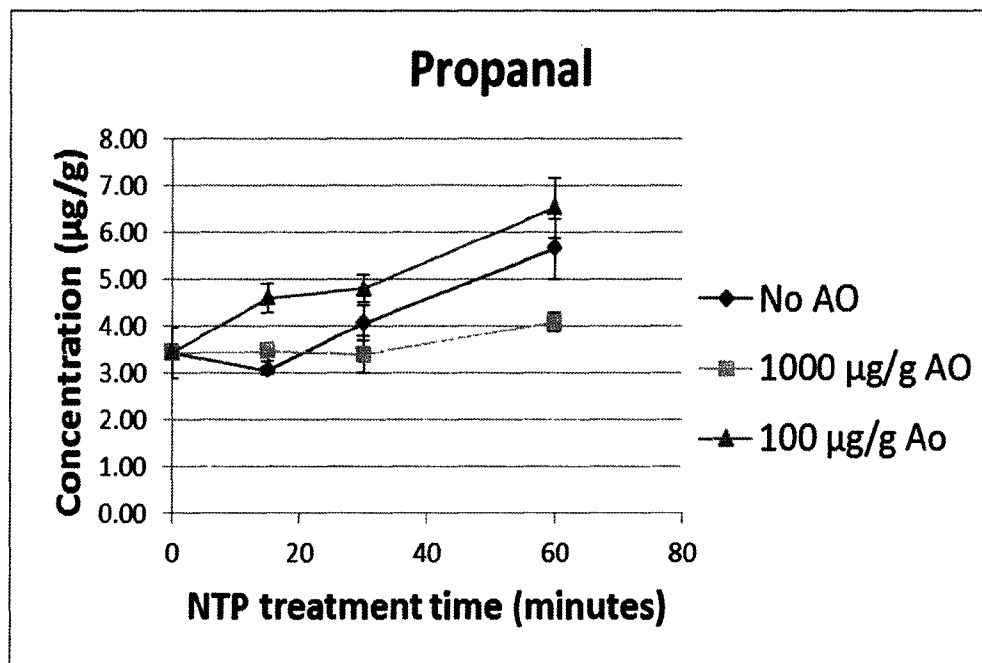


Figure 2c

a)



b)

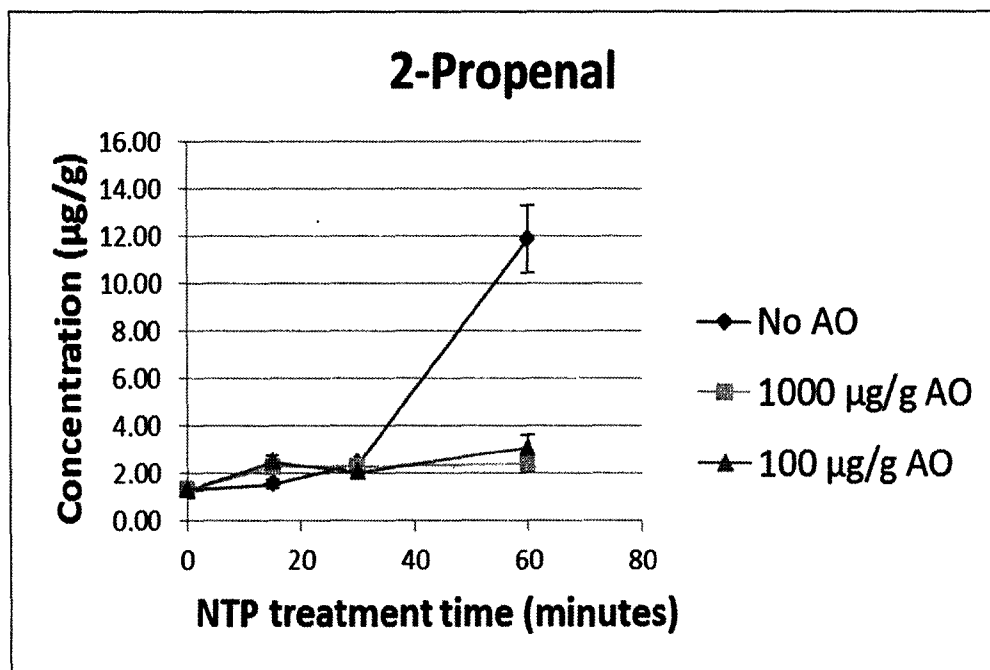


Figure 2c (continued)

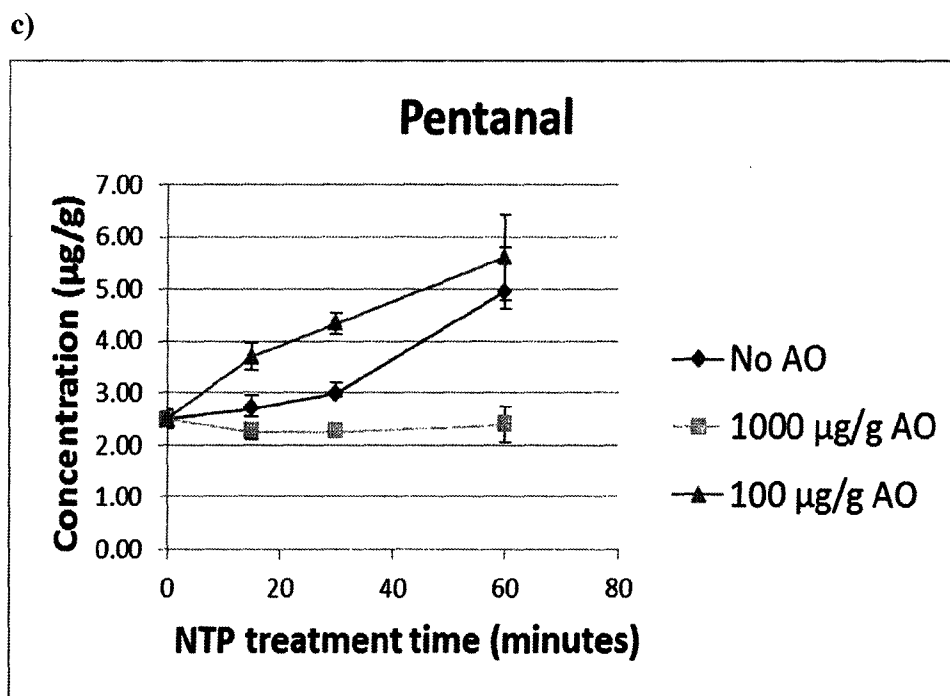
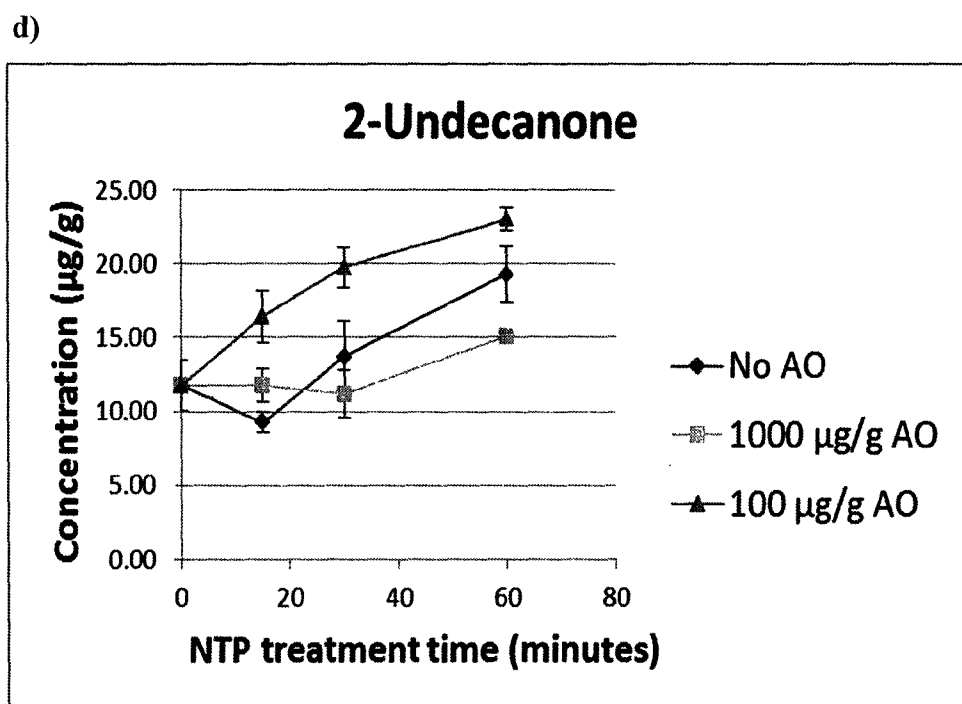
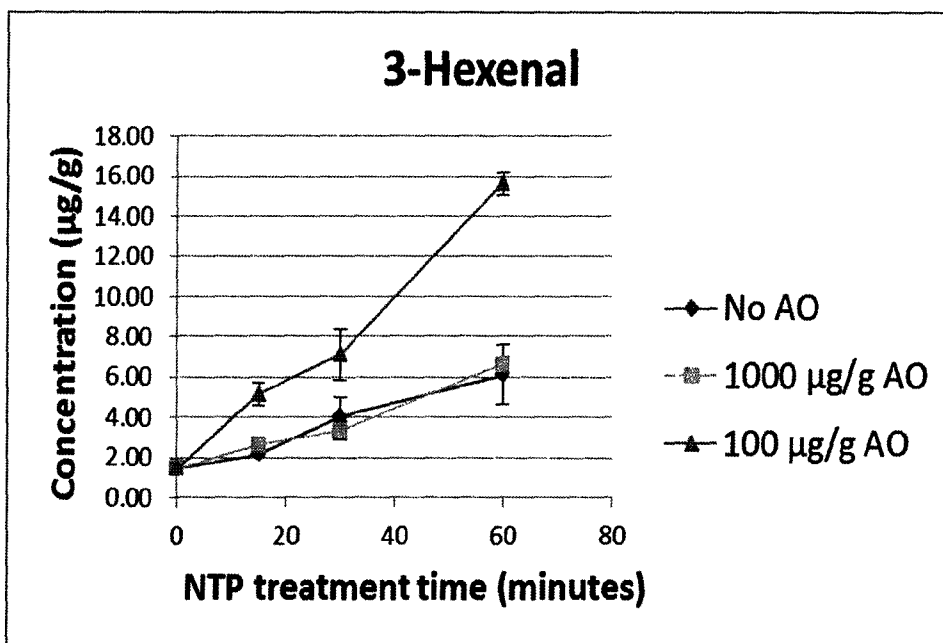


Figure 2c (continued)

e)



f)

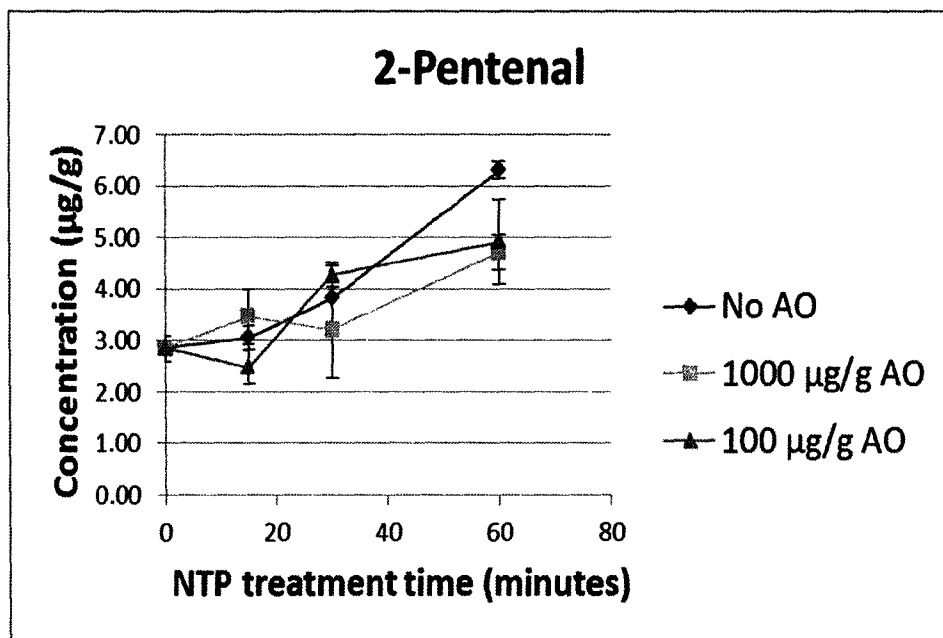
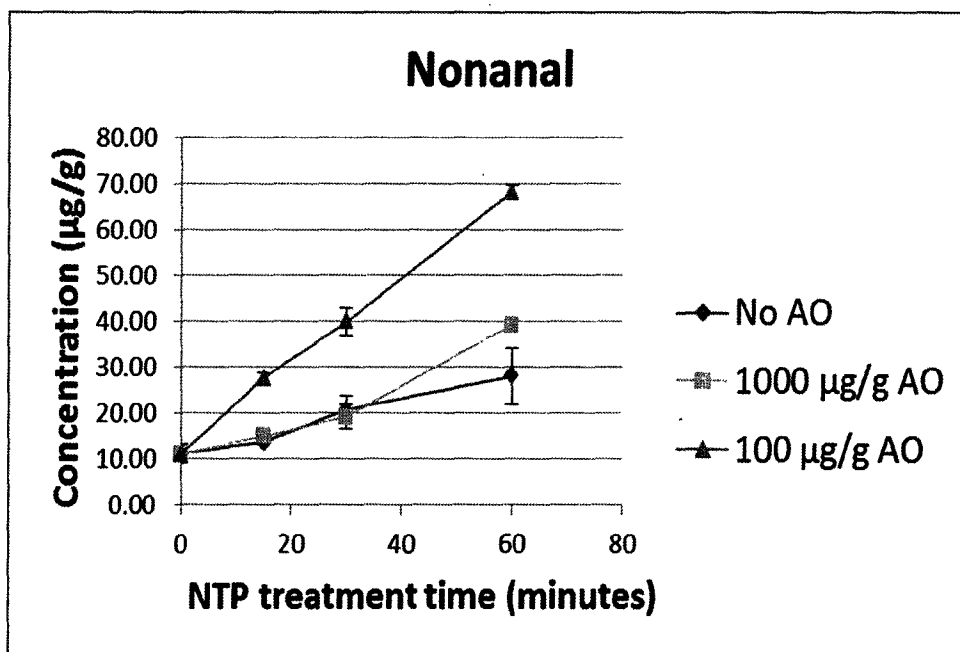


Figure 2c (continued)

g)



h)

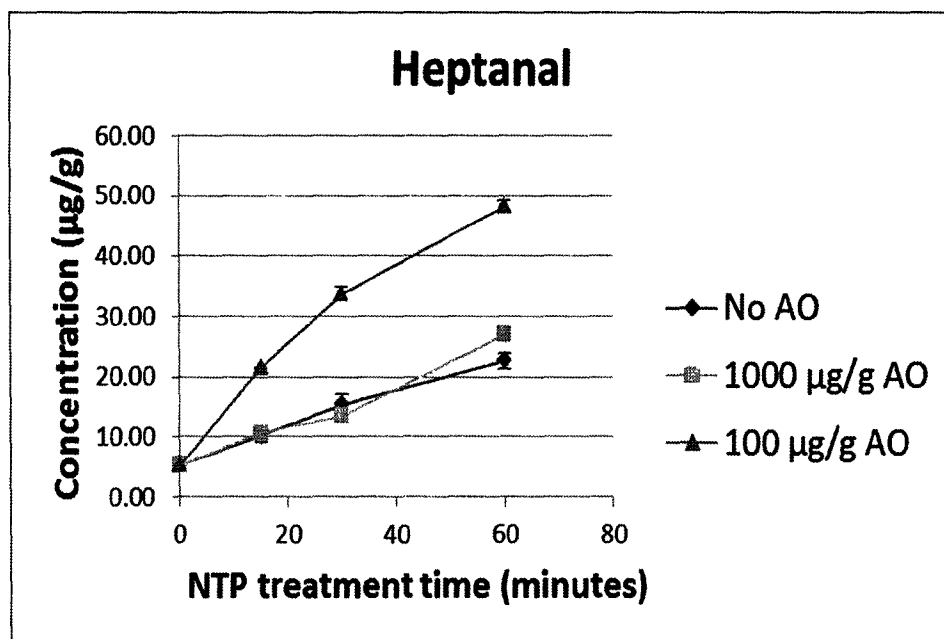


Figure 2c (continued)

i)

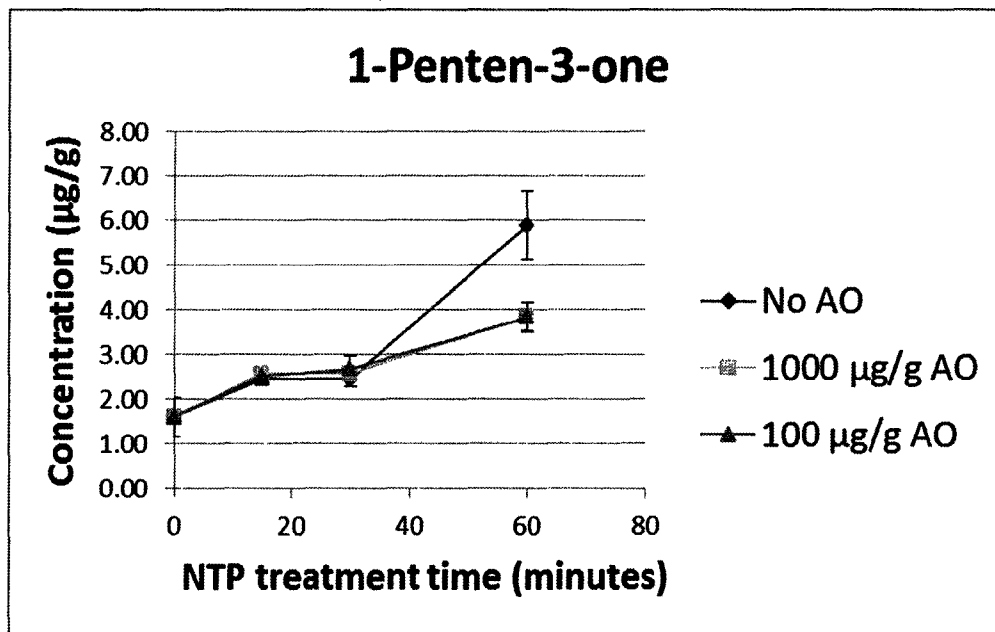
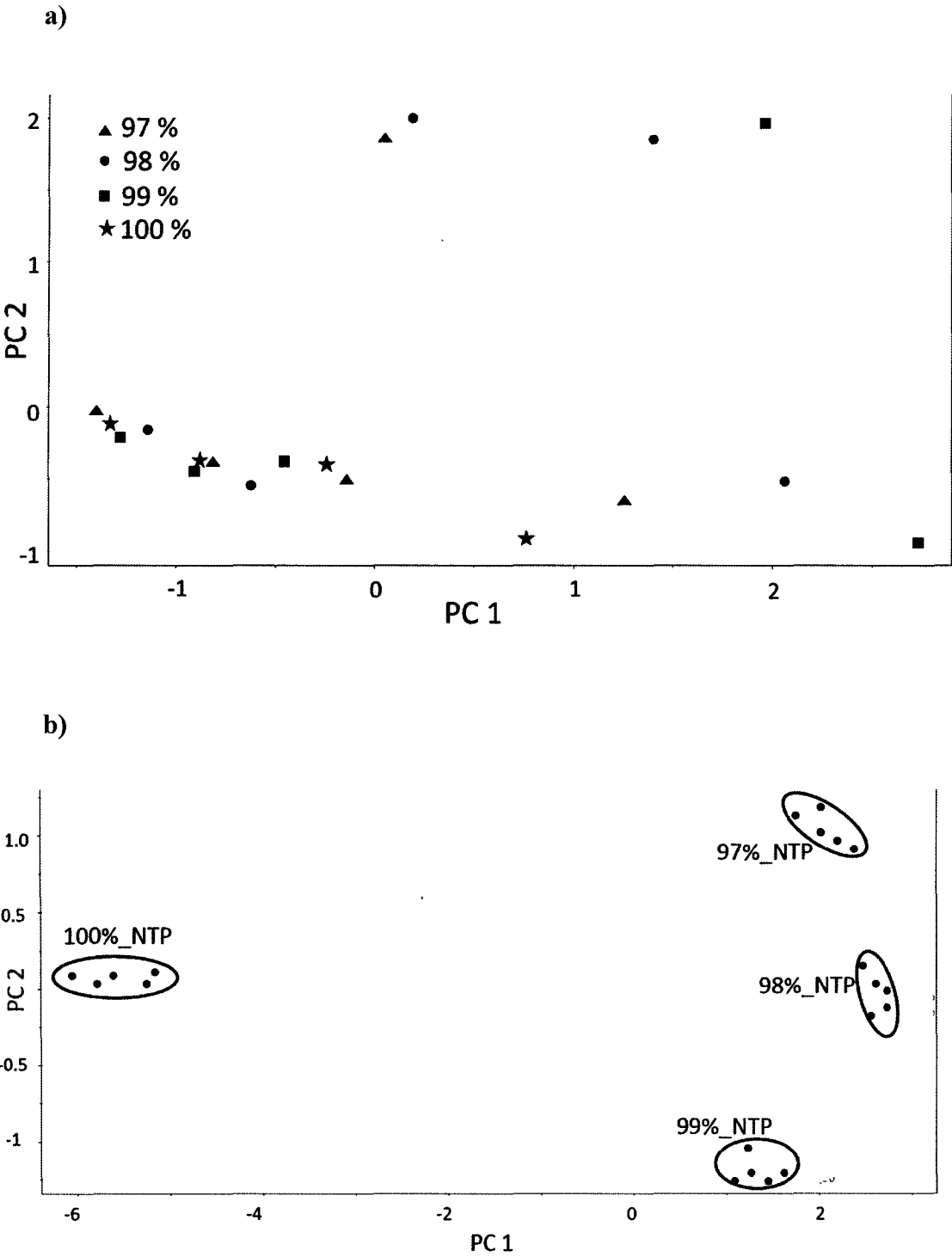


Figure 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/BE2017/000021

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/00 G01N1/22 G01N33/03
ADD.

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)
G01N

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

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

EPO-Internal, FSTA, WPI Data, INSPEC, COMPENDEX, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>VANDAMME JEROEN ET AL: "Controlled accelerated oxidation of oleic acid using a DBD plasma: Determination of volatile oxidation compounds", FOOD RESEARCH INTERNATIONAL, vol. 79, 27 November 2015 (2015-11-27), pages 54-63, XP029375509, ISSN: 0963-9969, DOI: 10.1016/J.FOODRES.2015.11.028 page 2 - page 4; figure 1 ----- -/--</p>	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

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"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

8 August 2017

Date of mailing of the international search report

16/08/2017

Name and mailing address of the ISA/

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Authorized officer

Joyce, David

INTERNATIONAL SEARCH REPORT

International application No
PCT/BE2017/000021

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHMID S ET AL: "Degradation of volatile organic compounds in a non-thermal plasma air purifier", CHEMOSPHERE, PERGAMON PRESS, OXFORD, GB, vol. 79, no. 2, 1 March 2010 (2010-03-01), pages 124-130, XP026934914, ISSN: 0045-6535, DOI: 10.1016/J.CHEMOSPHERE.2010.01.049 [retrieved on 2010-02-18] page 125 - page 126 -----	1-37
A	VANDAMME JEROEN ET AL: "Critical evaluation of non-thermal plasma as an innovative accelerated lipid oxidation technique in fish oil", FOOD RESEARCH INTERNATIONAL, vol. 72, 31 March 2015 (2015-03-31), pages 115-125, XP029240316, ISSN: 0963-9969, DOI: 10.1016/J.FOODRES.2015.03.037 cited in the application the whole document -----	1-37
A	GIUSEPPE PROCIDA ET AL: "Relationships between volatile compounds and sensory characteristics in virgin olive oil by analytical and chemometric approaches : Volatile compounds and sensory characteristics of olive oil", JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE, vol. 96, no. 1, 15 January 2016 (2016-01-15), pages 311-318, XP055391373, GB ISSN: 0022-5142, DOI: 10.1002/jsfa.7096 page 312, right-hand column -----	1-37
A	WO 2006/027690 A2 (DEVITTORI CARLO [CH]) 16 March 2006 (2006-03-16) abstract -----	3-5
X	DD 242 007 A1 (AKAD WISSENSCHAFTEN DDR [DD]) 14 January 1987 (1987-01-14) page 1 - page 2; figure 1 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/BE2017/000021

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006027690	A2	16-03-2006	NONE

DD 242007	A1	14-01-1987	NONE
