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SCIENTIFIC EVALUATION REPORT FOR THE OLIVE OIL "THE GOVERNOR"

In March 2014 we received in our laboratory a sample of olive oil with the brand name "The Governor" originating from the area of St. Matthaios, Corfu, Greece from the variety Lianolia Kerkyras and which was produced between November 2013 and January 2014.

The sample was analyzed at the laboratory of Pharmacognosy and natural products Chemistry of the faculty of Pharmacy of the Athens University under my responsibility based on a validated method that has been published in the Journal of the American Chemical Society J. Agric Food Chem., 2012, 60 (47), pp 11755-11703 and J. Agric Food Chem., 2014, 62 (3), 600-607.

Using this method we have analyzed more than 700 olive oil samples from the 2010-2011, 2011-2012, 2012-2013 and 2013-2014 periods. From these samples, 150 originate from countries outside Greece and specifically California, Italy, Spain, Croatia, Tunisia, Cyprus and a few samples from France, Argentina, Chile, Morocco, Israel. The rest come from all over Greece. In the archive of our laboratory we have data from olive oil samples coming more than 30 different varieties of olives.

The analyses of the above mentioned olive oil samples have been performed in Athens and in the University of California (UC Davis Olive center) under my supervision and for all of them we keep a detailed archive.

The chemical analysis of the olive oil "The Governor" showed the following results:

Oleocanthal: 680 mg/Kg

Oleacein: 350 mg/Kg

Oleuropein aglycon: 53 mg/Kg

Ligstroside aglycon: 59 mg/Kg

Total hydroxytyrosol derivatives: 403 mg/Kg

Total tyrosol derivatives: 739 mg/Kg

Oleocanthal+Oleacein (D1 index): 1030 mg/Kg

Total of analysed compounds (D3 index): 1142 mg/Kg

Comparing these results with the olive oil database results recorded in the archive of the University of Athens we are in the position to certify the following:

1. The oleocanthal concentration is 7 times higher than the average of the samples of Greek olive oils (99 mg/Kg) that were included in the study conducted at the University of Athens. The oleacein concentration is also 7 times greater than the average (48 mg/Kg).
2. The sum of Oleocanthal and Oleacein (D1 index), which has been proposed as an indicator of the health protecting properties of olive oil (there is extensive analysis on the importance of D1 index in the journal of the American Oil Chemical Society: Inform 04/2013; 24 (4): 266-270) **showed the highest value (1030 mg/Kg) compared to all the oils that have been analyzed in the 2013-2014 period.** Also this value was the highest among all the commercially available bottled oils from all the countries that have been recorded since 2009. So far we have recorded only one sample with higher value but it was not commercially available (relative tables of results have been published in J. Agric. Food Chem., 2014, 62 (3), 600–607).
3. The oleocanthal concentration (680 mg/Kg) **was the highest among all the commercially available bottled oils that we have recorded since 2009** and the third highest including the non-commercially available samples.
4. The oleacein concentrations was the second highest we have recorded for 2013-2014 and the 7th highest throughout the archive since 2009.
5. The sum of all the analyzed ingredients (D3 index = 1142 mg/Kg) was the second highest for the period 2013-2014, the second highest among all commercially available samples of Greek origin since 2009 and the sixth highest in the entire archive (from all countries, commercially or not available)

HEALTH CLAIM

1. The daily consumption of 20 g of the analyzed olive oil sample provides >5 mg of hydroxytyrosol, tyrosol or their derivatives and consequently the oil belongs to the category of oils that protect the blood lipids from oxidative stress according to the Regulation 432/2012 of the European Union.
2. The total hydroxytyrosol derivatives are 61% higher than the limit of the European regulation which makes sure that 18 months after harvesting the oil will still exceed the limit provided keeping in place away from high temperature and direct sunlight. In our laboratory we have performed aging studies for olive oil and we have shown that olive oils with D1 index > 200 in the time of bottling, after 18 months will present a reduction in the polyphenols level by 30%
3. It should be noted that oleocanthal, oleacein and oleuropein aglycon present important biological activity and they have been related with anti-inflammatory, antioxidant, cardioprotective and neuroprotective activity.

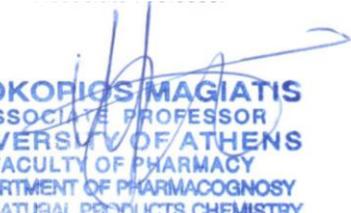
OVERALL EVALUATION

Based on the above presented results we can certify that it is an extremely rare extra virgin olive oil that stands out from the usual oils due to its high content of specific polyphenols which have major health protecting properties.

It is an oil which is highly recommended to all consumers looking for olive oil with enhanced properties for health protection.

This oil has a particularly pungent and bitter taste that is characteristic of the polyphenols which it contains. It is pleasantly consumed with foods that have a neutral taste.

The responsible for the scientific evaluation report



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ANNEX

Pharmacological and biological properties of the measured compounds

A summary of the available scientific data for the measured compounds is following:

In total, oleocanthal, oleacein, oleuropein aglycon, ligstroside aglycon present important biological activity and they have been related with anti-inflammatory, antioxidant, cardioprotective and neuroprotective activity.

Biological properties of oleocanthal

Oleocanthal is the dialdehydic form of the ligstroside aglycon without the carboxymethyl group (Mateos R. et al., 2004) which has been related with the “bitter and burning” sensation on the throat, mainly on the base of the tongue that occurs when tasting extra-virgin olive oil.

The pharmacological actions of oleocanthal are the following:

- Action against Alzheimer’s disease

According to scientific research, it has been proved that the soluble oligomers of amyloid-b1-42 peptide act as the basic neurotoxin in the Alzheimer’s disease. As a result, compounds that can modify the formation of these oligomers, referred to as ADDLs, are possible to have therapeutic effects on the AD.

Oleocanthal is of particular interest because of its ability to perturb this particular oligomerisation and reduce the pathogenesis of AD by protecting, at the same time, the neurones from ADDLs’ effects on the synapse pathology. (Pitt J. et. al., 2009). Oleocanthal is also able to inhibit the tau-fibrils formation by modifying covalently the PHF-6 peptide which is of vital importance to their formation. This leads to neurofibrillary tangles which are inherently linked to neurodegenerative diseases as the AD.

The two aldehyde groups of oleocanthal react with the structural unit of lysine to form a complex through the Schiff base formation reaction on the PHF6 peptide, affecting significantly the accumulation of T (tau) proteins in neurofibrils. (Li W. et al., 2009).

It has also been found that oleocanthal interacts with the tau-441 protein causing the stabilization of its secondary structure conformation, thus no further conformation can take place. (Monti M.C et al., 2012)

By examining the oleocanthal action on cell culture, but also on guinea pigs’ brain, it has been discovered that oleocanthal is possible to successfully remove amyloid- β (A β) from the brain, through an increase in the expression and the activity of P-glycoproteins (P-gp) and LDL lipoproteins receptors. LDL lipoproteins bind with proteins-1 (LRP1), which are big A β transfer proteins to the blood-brain barrier.

So, it has been discovered that oleocanthal increases the P-gp and LRP1 activity and consequently the A β removal increases.

As a result, the following conclusion is extracted: potential decrease in the risk of developing AD, which is linked to the extra-virgin oil consumption, could be due to the A β removal reinforcement from the brain. (Abuznait A.H., 2013)

- Action against osteoarthritis

Osteoarthritis is a disease whose progression is characterized by increased nitrogen monoxide (NO) production which involves degradation of articulate cartilage.

Recently, oleocanthal isolated from extra-virgin olive oil was found to present anti-inflammatory action similar to that of ibuprofen, a non-steroid anti-inflammatory drug used widely in the therapeutic treatment of common inflammatory diseases.

Oleocanthal and its derivatives reduce the lipopolysaccharides-induced synthesis of NO in cartilage cells without significant effect on the cell viability.

Moreover, oleocanthal intervenes in prostaglandin synthesis through the inhibiting action it exerts on the cyclooxygenase enzymes. (Cicerale S. et al., 2012)

Therefore, oleocanthal can potentially be used as a therapeutic weapon in the treatment of inflammatory degenerative diseases, both in the cure of rheumatoid arthritis and in that of osteoarthritis. (Iacono et al., 2010)

- Inhibition of cyclooxygenase enzymes COX-1 και COX-2

Oleocanthal inhibits cyclooxygenase enzymes in the biosynthetic path of prostaglandins; the latter being inflammatory factors. (Iacono A., 2010)

Oleocanthal has been proved to inhibit cyclooxygenase enzymes in a dose-related manner, imitating the anti-inflammatory action of ibuprofen. (Beauchamp G.K. et al., 2005)

The cyclooxygenase enzymes 1 and 2 (COX 1 and COX 2) are responsible for the transformation of arachidonic acid into prostaglandins and thromboxanes, which are produced as a response to inflammatory or toxic stimuli.

Cyclooxygenase 1 and 2 can be harmful to the human body.

Especially, research made both on humans and on animals, has showed that COX 2 is involved in the pathogenesis of different types of cancer (Harris R.E. et al., 2003, Boland G. et al., 2004, Subbaramaiah K. et al., 2002) and may also play a role in atherosclerosis. (Chenevard R. et al., 2003).

Findings presented by Beauchamp G.K. et al., prove that oleocanthal not only imitates the mechanism of action of ibuprofen, but also presents higher activity compared to the latter, in inhibiting the action of COX 1 and COX 2 in equimolar concentrations.

For example, a concentration of 25 μ M of oleocanthal inhibited the activity of COX up to 41-57%, whereas, a concentration of 25 μ M of ibuprofen inhibited only 13-18% of the COX activity.

Furthermore, it has been suggested that long-term intake of small quantities of oleocanthal from extra-virgin olive oil consumption, are in part responsible for the low incidence of diseases associated with a Mediterranean diet.

It is known that low long-term doses of ibuprofen and of other COX inhibitors, like aspirin, have significant benefits on human health as far as the prevention from developing cancer (e.g. rectum and breast cancer) (Garcia-Rodriguez L. A. & Huerta-Alvarez C., 2001, Harris R. E. et al., 2006) and cardiovascular disease (Hennekens C.H., 2002) is concerned.

As a result, long-term consumption of extra-virgin olive oil rich in oleocanthal may contribute to reduction in the incidence of the above diseases. (Cicerale S. et al., 2012)

- **Anti-tumor action / Action against cancer**

Oleocanthal is able to inhibit the proliferation and metastasis of tumor cell lines of human mammary and prostate epithelial cells, and has shown anti-angiogenic activity.

It blocks the phosphorylation of c-Met kinase in vitro with IC_{50} 4,8 μ M, and may, therefore, have a therapeutic use in the control of malignancies.

It also inhibits proliferation, metastasis and invasion of MCF7, MDA-MB231 and PC-3 tumor cell lines of the breast and the prostate, respectively.

- **Controls skin ageing**

It prevents excessive proliferation of keratinocytes in stress situations due to environmental reasons, by stimulating the synthesis of skin lipids, and genes that are directly related to the differentiation among cells. (Estanove C. et al., 2009).

- **Antimicrobial activity**

Oleocanthal, as proven, cannot be hydrolyzed in the stomach and thanks to this helps inhibit the bacterium *Helicobacter pylori* growth, which is responsible for developing peptic ulcer and certain types of cancer. (Romero C. et al., 2007)

Biological properties of oleacin

Oleacin is a substance with pharmacological actions similar to those of oleocanthal.

- **Anti-oxidant activity**

Oleacin, unlike oleocanthal, has a significant action in that it protects human cells from oxidative stress to a greater extent, even when compared to hydroxytyrosol. (Pavia-Martins F. P. et al., 2009).

- **Angiotensin Converting Enzyme (ACE) inhibitor**

ACE inhibition induces vasodilation; thus, oleacin could potentially be used in the treatment of hypertension and heart failure. (Hansen K. et al., 1996, Somova L.I. et al., 2003)

- **Αντιγηραντική δράση**

- Anti-ageing action

Oleacin is a strong anti-oxidant and so it can prevent LDL oxidation, oxidative stress and oxidative damage on DNA.

Hematopoietic endothelial progenitor cells (EPC) are the basic mechanism through which damaged blood vessels are repaired.

These cells are also particularly sensitive to oxidative stress.

Oleacin reduces cell ageing induced by angiotensin II and inhibits the formation of reactive oxygen species (ROS).

This results in a decrease in nitrotyrosine and in oxidative damage on DNA.

Oleacin augments the angiogenic potential of the hematopoietic endothelial progenitor cells *in vitro*.

Oleacin can, therefore, protect the EPCs from oxidative stress caused by angiotensin II. (Parzonko A. et al., 2012)

- Heart-protective activity

Oleacin inhibits myeloperoxidase release from neutrophils, which can explain the important role of olive oil in the prevention of cardiovascular diseases. (Czerwińska M. et al., 2012–a)

- Anti-inflammatory action

Oleacin is strong lipooxygenase (LOX) enzyme inhibitor taking part in the inflammation procedure.

Biological properties of the monaldehydic form of the oleuropein aglycon

Chemically, it is classified under ortho-diphenols category, which are a bioactive part in the mechanism of protection of the olive tree fruit from insect attacks and pathogenic bacteria. (Bianco A.D. et al., 1999).

It is also known for its antioxidant action which is comparable to oleuropein.

Moreover, there is evidence that it can prevent further cell proliferation in breast cancer. (Pérez-Trujillo M. et al., 2010).

- Protection from diabetes

Amylin (IAPP) was discovered in 1987, when isolated from amyloid deposits, taken post mortem from pancreas of patients with type II diabetes.

It is a peptide hormone consisting of 37 amino acids, which is located along with insulin in the secretory granules of the pancreas beta-cells and is secreted in response to trophic stimuli and other secretagogues.

Tests on humans have demonstrated that concentrations of insulin and amylin in plasma raised and lowered parallel, following meals.

The pancreatic amylin deposition is a hallmark of diabetes mellitus type II and there is ample evidence that amylin oligomers exhibit cytotoxic action to the pancreas beta-cells.

The oleuropein aglycon prevents increasing the concentration of amylin in the human body.

It has also been shown that amylin aggregates, grown in the presence of oleuropein aglycon, cannot interact with the cell membrane and destabilize it.

As a result, there is no cytotoxicity in beta-cells. (Rigaccia S. et al., 2010)

- Protection from the Alzheimer's disease

The oleuropein aglycon has been found to be one of the substances responsible for being involved in the protection of the central nervous system from degeneration caused by Alzheimer's disease, by reducing the levels of beta-amyloid, as it has been demonstrated in guinea pigs. (PLOSone 2013, DOI: 10.1371/journal.pone.0071702).