Ελληνικό Φόρουμ για την Επιστήμη και Τεχνολογία Λιπιδίων (Greek Lipid Forum)

Ελληνικό Φόρουμ για την Επιστήμη
και Τεχνολογία Λιπιδίων
(Greek Lipid Forum)

5ο Πανελλήνιο Συνέδριο

«ΣΥΓΧΡΟΝΕΣ ΤΑΣΕΙΣ ΣΤΟΝ ΤΟΜΕΑ ΤΩΝ ΛΙΠΙΔΙΩΝ»

Αθήνα 29 Μαρτίου 2013
Χαροκόπειο Πανεπιστήμιο

Βιβλίο Περιλήψεων

Euro Fed Lipid
European Federation for the Science and Technology of Lipids

Ελληνικό Φόρουμ για την Επιστήμη και Τεχνολογία Λιπιδίων (Greek Lipid Forum)

5ο Πανελλήνιο Επιστημονικό συνέδριο

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Χαροκόπειο Πανεπιστήμιο

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Πίνακας Περιεχομένων

Α. Περιλήψεις προφορικών παρουσιάσεων

1η Συνεδρία: Αναλυτικές τεχνικές. Εξελίξεις στις τεχνολογίες λιπαρών. Λειτουργικά συστατικά

Προεδρείο: Σ. Αντωνοπούλου
Φ. Νταής

O-1	Structure and morphology of the emulsifier polyglycerol ricinileate (PGPR)	**P. Dais**, A. Orfanakis, E. Hatzakis, K. Kanaki, S.A. Pergantis, A. Rizos
O-2	FT-MIR/ATR monitoring of virgin olive oil oxidative stability under mild storage conditions	N. **Nenadis**, I. Tsikouras, P. Xenikakis, M. Z. Tsimidou
O-3	Detection of virgin olive oil adulteration by synchronous fluorescence spectroscopy	A. Papadochristopoulos, M.A. Poiana, E. Alexa, G.A. **Mousdis**, C. A. Georgiou
O-4	Ultrasound assisted extraction of a lipid fraction enriched in squalene from industrial wine lees	E. **Naziri**, F. Mantzouridou, M. Z. Tsimidou
O-5	Capillary rise in porous media to set rejection criteria for reused fried oils	J. S. **Lioumpas**, A. Zamanis, Th. Karapantsios
O-6	Determination of 4 major PAHs using Reverse Phase Liquid Chromatography	G. Siragakis, M. Christofakis
O-7	Bioactive microconstituents of wild edible mushrooms from the island of Lesvos, Greece	A. E. **Yanni**, N. Kalogeropoulos, G. Koutrotsios, M. Aloupi

Δομή και μορφολογία του γαλακτωματοποιητή εστέρας της γλυκερόλης με ρισινελαϊκό οξύ

Structure and morphology of the emulsifier polyglycerol ricinileate (PGPR)

P. Dais, A. Orfanakis, E. Hatzakis, K. Kanaki, S.A. Pergantis, A. Rizos

Three different analytical techniques, namely NMR spectroscopy, mass spectrometry and dynamic light scattering, were used to unravel the structure and morphology of polyglycerol polyricinoleate (PGPR). This material is used as an emulsifier in the preparation of chocolate and other confectionary products. The use of 1D and 2D NMR techniques led to the distinction of two separate entities in commercial ricinoleic acid (RA) and PGPR samples, namely the monomeric and oligomeric RA (estolides). ^{1}H and ^{13}C spectra of PGPR confirmed the presence of polyglycerol moieties of various lengths being esterified by RA and estolides and to a lesser extent by oleic and linoleic acids. ^{13}C-NMR DOSY experiments demonstrated the occurrence of several species in PGPR. Electrospray Ionization and tandem Mass Spectrometry succeeded in identifying the presence of over 30 glycerol/polyglycerol species containing n glycerol moieties with n = 1–6 esterified by monomeric and oligomeric RA molecules. Dynamic light scattering contributed to the characterization of PGPR morphology. The PGPR mixture contains relatively small-sized entities (monomers, dimmers, trimmers) and larger aggregates resulted from chain association. The percentage of larger aggregates is minimal compared to small-sized species.

Παρακολούθηση της οξειδωτικής κατάστασης παρθένων ελαιολάδων κατά την αποθήκευση σε ήπιες συνθήκες με την τεχνική FT-IR/ATR

FT-MIR/ATR monitoring of virgin olive oil oxidative stability under mild storage conditions

N. Nenadis, I. Tsikouras, P. Xenikakis, M. Z. Tsimidou

The aim of the present study was to exploit the technique Fourier Mid-IR spectroscopy with Attenuated Total Reflectance (ATR) as a means to extract information related to the oxidative status of virgin olive oil (VOO) under mild storage conditions. Eleven VOOs obtained from the regional union of Lassithi (Crete, Greece) collected directly form the three phase decanter were stored in the dark (25 ^{o}C) in glass bottles with a 10% headspace for 12 months. Measurements of free acidity, wax values, total sterols, $K_{232/270}$ indices and FTIR spectra were obtained periodically (0, 6 and 12 months). After the 12 months all values satisfied official limits set by EC Regulation (1989/2003). Changes in intensity values at 3470 cm^{-1} (hydroperoxide formation) and in values of various intensity ratios relevant to the degree of VOO unsaturation (A3006/2924, A3006/2853, A3006/1746, A3006/1465, A3006/1163, A1118/1097, A2853/1746, A2853/1417, A2853/1163, A2853/1118, A2853/1097 and A2853/723) did not provide more information than the physicochemical criteria when examined individually. Application of chemometrics, namely Principal component - discriminant analysis of selected intensity values (2924, 2853, 1746, 1465, 1163, 1118, 1097 and 723 cm^{-1}) led to a 100%, 91% and 54.5% correct classification for the 12, 6 and 0 months stored samples. Better classification of the fresh ones (73 - 91%) was achieved when the selected chemometric approach was employed to the second derivatives of various spectral ranges. Frequencies assigned to stretching and/or bending of -C-O and -CH_2- groups (1020-1260 cm^{-1}) produced the best sample discrimination. Present findings add to the usefulness of FT-IR/ATR as a rigorous and low cost technique for olive oil quality control. Even so, systematic inter-laboratory studies are required before it can be considered as a robust tool in olive oil analysis.

Detection of virgin olive oil adulteration by synchronous fluorescence spectroscopy

A. Papadochristopoulos, M.A. Poiana, E. Alexa, G.A. Mousdis, C. A. Georgiou

Adulteration of extra virgin olive oil with corn oil is a major issue for the olive oil industry. In this presentation, the potential of total synchronous fluorescence (TSyF) spectra to differentiate virgin olive oil from corn oil and synchronous fluorescence (SyF) spectra combined with multivariate analysis to assess the adulteration of virgin olive oil are demonstrated. TSyF spectra were acquired by varying the excitation wavelength in the region 300-800 nm and the wavelength interval (Δλ) in the region from 10 to 100 nm. TSyF contour plots for corn oil, in contrast to virgin olive oil, show a wider fluorescence region (Fig. 1.). Different virgin olive oil samples were adulterated by corn oil at varying levels (0.5-95%) resulting in more than hundred mixtures. Partial least square regression model was used for quantification of the adulteration. This technique is useful for detection of corn oil in virgin olive oil at low levels in just two and a half minutes.

Acknowledgment

This work was financially supported by the Greek General Secretariat for Research and Technology through a bilateral Romania-Greece project.

Παραλαβή κλάσματος λιπιδίων εμπλουτισμένο σε σκουαλένιο από τις οινολάσπες

Ultrasound assisted extraction of a lipid fraction enriched in squalene from industrial wine lees

E. Naziri, F. Mantzouridou, M. Z. Tsimidou

The exploitation of wastes and by-products produced by the winery industry seems to be a cost-effective and an environmental friendly investment. A number of valuable components can be recovered from yeast lees (e.g. tartaric acid, b-1,3-glucans), whereas up to now, there is no literature data concerning recovery of squalene. Squalene is a bioactive compound of great importance, finding application to food, pharmaceutical and cosmetic industry. Nowadays, increasing is the interest in establishing novel squalene sources as the conventional ones are rather limited.

The aim of the present study was the selective ultrasound assisted extraction of a lipid fraction enriched in squalene, from lees using the food-grade solvent, *n*-hexane. A central composite design was applied to select ultrasound operational conditions. The potential influence of the two independent factors, namely duration (1-29 min) and duty cycles (active intervals 0.3-1.0 s) of sonication to the squalene yield (SQY) (mg/Kg of lipid extract) and the lipid yield (LY) (%, w/w of dry lees) were examined. Chromatographic methods were applied to monitor squalene content and to characterize composition of the lipid extracts.

Under the optimum operational conditions, SQY and LY were found to be 20390 ± 1335 mg SQ/Kg lipid extract (or 0.6 g/Kg dry lees) and 2.5 ± 0.2 % dry lees, respectively. The examined lipid fraction contained 0.6 g SQ/Kg dry biomass, which was comparable to those of recently examined wastes and by-products as potential sources (e.g. 0.2-0.35 g SQ/Kg of dry olive pomace and 0.06 g SQ/Kg olive leaves).

Κριτήρια απόρριψης επαναχρησιμοποιούμενων τηγανέλαιων με χρήση της τριχοειδούς αναρρίχησης σε πορώδη μέσα

Capillary rise in porous media to set rejection criteria for reused fried oils

J. S. Lioumpas, A. Zamanis, Th. Karapantsios

The intense and complex heat and mass transfer processes during deep fat frying result in significant oil degradation which imposes oil replenishment in sequential frying batches. The determination of the exact instant that frying oil must be replenished is a major concern for avoiding possible health risks but also for estimating the cost of fried foods in food industry and catering applications. This work investigates the potential of setting the fried oil rejection criteria by employing the phenomenon of capillary rise of oil into a porous medium. To achieve this goal, wicking patterns (oil penetration rate and oil front shape versus time) of both fresh and prolonged fried oils are optically registered at six different paper sheets used as porous media. Four of them are double-ply towel papers whereas the other two are single-ply chromatographic papers. Wicking tests are performed at 20^{o}C and 30^{o}C. The data of the present study show that the type of paper affects seriously the wicking patterns. Double-ply papers present high oil penetration rates but very irregular oil front shapes whereas single-ply papers yield lower oil penetration rates but pretty flat oil fronts. Furthermore, it is found that only under certain conditions the penetration rates obey the well known Lucas – Washburn equation. A discussion is made on the phenomena that take place during wicking of oil into paper which may cause deviations from the Lucas – Washburn equation. A semi-empirical model is proposed to describe the above deviations by incorporating the effect of time evolving pore sizes.

Προσδιορισμός των 4 κύριων Πολυκυκλικών Αρωματικών Υδρογονανθράκων με τη χρήση Υγρής Χρωματογραφίας Αντίστροφης Φάσης

Determination of 4 major PAHs using Reverse Phase Liquid Chromatography

G. Siragakis, M. Christofakis

PAHs are a group of organic compounds formed mainly from the incomplete combustion of various fuels, and consist of at least two fused benzene rings. Many PAHs are suspected to be cancerogenic and mutagenic. Due to their toxicity, persistence and widespread distribution, PAHs are a major environmental concern. In 2008, an expert's opinion from the European Food Safety Authority (EFSA) questioned this statement and proposed the sum of the 4 PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene) as a more suitable indicator for the occurrence of PAHs in food. Next to the already existing maximum levels for benzo[a]pyrene (Regulation 1881/2006), the latest Regulation (EU) 835/2011 lists the maximum levels for the so-called PAH4.

This work includes method development and validation for quantitative determination of these four PAHs, benzo [a] pyrene, benzo [a] anthracene, benzo [b] fluoranthene, chrysene and their sum in crude and refined edible oils and fats by Reverse Phase Liquid Chromatography using Fluorescence detector (Agilent 1200 HPLC-FLD). The method was based on the standard ISO 15302:2010 which even though specifies a method for the determination of benzo[a]pyrene, it was modified to meet the needs of the analysis; determination of the other 3 hydrocarbons as well. Regulation (EU) 836/2011 sets six method performance criteria (Specificity, Reproducibility, Repeatability, Recovery, LOQ, LOD) all of which are met in our method. The method has been accredited and is used for routine analysis.

Βιοδραστικά μικροσυστατικά άγριων βρώσιμων μανιταριών της Λέσβου

Bioactive microconstituents of wild edible mushrooms from the island of Lesvos, Greece

A. E. Yanni, N. Kalogeropoulos, G. Koutrotsios, M. Aloupi

Mushrooms have been a perennial component of human diet, consumed both as part of the normal diet and as a delicacy, due of their texture, taste and aroma. In Greece, wild edible species comprise an important ingredient for the traditional cuisine and gastronomy. Mushrooms are functional foods with nutritional and nutraceutical properties and source of beneficial bioactive compounds.

In the present work, we report the fatty acids, sterols, individual polyphenols and terpenic acids in wild edible mushrooms, namely *Lactarius deliciosus, Lactarius sanguifluus, Lactarius semisanguifluus, Russula delica,* and *Suillus bellinii*, collected from several sites in the island of Lesvos, Greece.

All analyses were carried out by GC-MS. Up to 26 fatty acids were detected in mushroom lipids. Linoleic acid (C18:2ω6) predominated in *Russula* samples, linoleic and stearic (C18:0) acids in *Lactarius* species, while oleic (C18:1ω9) was the more abundant fatty acid in *S. bellinii;* palmitic acid (C16:0) was among the major fatty acids. Mushroom lipids also contained low amounts of elaidic acid (*trans*-oleic acid), conjugated linoleic acid and the highly unsaturated arachidonic (20:4ω6) and eicosapentaenoic (20:5ω3) acids. Ergosterol was the main among the sterols with concentrations from 9.2-18 mg/100 fw. When mushrooms are exposed to UV radiation ergosterol is converted to vitamin D_2 (ergocalciferol) in amounts that can be nutritionally significant. Vitamin D_2 from fungi and mushrooms serves as the only available dietary source of vitamin D for those who eat no animal products. Up to nineteen simple polyphenols and the triterpenic acids oleanolic and ursolic were determined in mushrooms extracts, the more abundant being *p*-OH-benzoic acid, *p*-OH-phenylacetic acid, *o*-coumaric acid, ferulic acid and chrysin.

The results are expected to contribute to the nutritional and chemical characterization of wild species making available a chemical inventory of wild edible mushrooms in order to both promote their consumption and preserve their habitats.

2η Συνεδρία: Ελαιόλαδο. Λίπη, έλαια και διατροφή

	Προεδρείο:	Π. Καλαϊτζής Β. Σινάνογλου
O-8	Safety and quality of Olive Oil in Greek market. Current status	K. Barberis, L. **Palilis**
O-9	Nutrition and health claims for Greek traditional foods: Reflections on a high olive oil diet	V. **Dilis**, E. Vasilopoulou, A. Trichopoulou
O-10	Effect of processing variables on olive oil quality characteristics process efficiency	E. Kitsios, P. Ioakeimidis, E.P. **Kalogianni**
O-11	The use of chloroplastic SNPs for the identification of cultivar origin of olive oil	P. **Kalaitzis**, I. Manolikaki, Ch. Bazakos, T. Spanos
O-12	Development of DNA-based Olive Oil Authenticicity Tests	D.P. **Kalogianni**, L . Boutsika, Ch. Bazakos, T. K. Christopoulos, P. Kalaitzis
O-13	Comparison of fatty acid composition of milk obtained from conventional and organic dairy sheep farms	S. Maragoudakis, T. **Massouras**, I. Hadjigeorgiou
O-14	The Greek national nutrition and health survey "HYDRIA": Method of implementation	E. **Valanou**, D. Oikonomidou, A. Androulidaki, I. Gkoufa, M. Kritikou, M. Pantzalis, P. Vidalis, I. Ziara, A. Naska, A. Trichopoulou

Ασφάλεια υγιεινή και τυποποιημένο ελαιόλαδο

Safety and quality of Olive Oil in Greek market. Current status

K. Barberis, L. Palilis

Olive oil, the basis of the Mediterranean Diet is considered to be one of the most popular national product of Greece. Greece enjoys the largest consumption of olive oil per capita, with the average Greek consuming more than 15 kg annually. Hellenic Food Authority (EFET) as National Competent Authority is responsible for planning and conducting official controls to olive oil marketed in the Greek market or intended to be export to other countries.

Official controls on olive oil can be divided in two groups: The first type of controls deals with the safety of olive oil. The presence of several contaminants in olive oil is being monitored every year. The contaminants being monitored include polycyclic aromatic hydrocarbons, pesticides residue, dioxins and PCBs, lead and phthalate esters.

On the other hand, official controls focus on the quality of olive oil. Commission Regulation (EEC) No 2568/91 on the characteristics of olive oil together with the Commission Implementing Regulation (EU) No 29/2012 on marketing standards for olive oil are the basis for these controls.

The results of the official controls conducted the last years that will be presented, verify that Greek olive oil complies with the safety and quality standards of European Union legislation and rightly holds the reputation of a quality product.

Ισχυρισμοί διατροφής και υγείας σε ελληνικά παραδοσιακά τρόφιμα: ο ρόλος του ελαιόλαδου

Nutrition and health claims for Greek traditional foods: Reflections on a high olive oil diet

V. Dilis, E. Vasilopoulou, A. Trichopoulou

Background: European Commission (EC) Regulation 1924/2006 provides the legal framework for the use of nutrition and health claims on foods in the European market, on the basis of scientific evidence. Although the Mediterranean diet has long been recognized for its nutritional quality and adequacy, individual Mediterranean traditional foods are commercially under-represented in comparison to novel and fast foods. Regulation 1924/2006 could endorse nutritionally rich foods, such as Mediterranean traditional foods and, thus, increase their availability in the European households.

Methods: One hundred and ninety four Greek traditional foods were selected from the Greek food composition tables, and their nutritional composition was evaluated on the basis of the thresholds for nutrition and health claims set by the EC. The following nutritional values, high or low as desired, were taken into account: energy, protein, total fat and fatty acids, sugars (mono + disaccharides), salt (sodium), dietary fibre, vitamins (B6, C, A, E, riboflavin, thiamin and β-carotene) and minerals (P, Mg, Fe, Zn, Ca, Cu and K).

Results: On the basis of the compositional data used, 1024 nutrition claims could apply to the 194 foods studied, with an average of about 5 nutrition claims per food. Of those nutrition claims, 529 were related to vitamins and minerals. Health claims could be considered in at least half as many occasions as nutrition claims for vitamins, minerals, protein, saturated-monounsaturated-polyunsaturated fatty acids and sodium. More than two thirds of the foods were high in monounsaturated fatty acids.

Conclusions: The European regulation on nutrition and health claims on foods could highlight basic characteristics of the Greek traditional diet i.e. the ample use of olive oil and nutrient dense plant foods such as vegetables. The application of this legislation may benefit small business operators involved in the production or promotion of traditional foods. It could also promote the health of European citizens by underlining the nutritional quality of these traditional foods and eventually increase their share in the common market.

Επίδραση παραμέτρων της διεργασίας παραγωγής ελαιολάδου στα ποιοτικά χαρακτηριστικά του ελαιολάδου και στην και την απόδοση της διεργασίας

Effect of processing variables on olive oil quality characteristics process efficiency

E. Kitsios, P. Ioakeimidis, E.P. Kalogianni

This work examines the effect of processing variables on olive oil quality and process efficiency. In order to better simulate actual processing conditions a small industrial scale (500 kg_{olives}/hr) olive oil production plant was used. The plant offers the possibility to accurately control and vary processing variables and therefore is suitable for experimental studies. All steps of olive oil extraction were examined: crushing, malaxation and olive oil separation in the decanter and centrifuge. Two crushing methods were examined: disc crusher and disc crusher combined with depitter. Furthermore, the effect of malaxation time and temperature were examined. In addition the feeding rate from the malaxer to the decanter was examined. Finally, special focus was given on the effect of water flow rate and water temperature added in the two-phase decanter and disc centrifuge. The olive oil quality characteristics examined were: acidity, peroxide value, K_{232}, K_{270}, ΔK, total phenol concentration, oil stability index. Moreover, the olive oil fatty acid profile was determined using gas chromatography, and olive oil sensory attributes were determined. The process characteristics examined were: olive oil production rate and olive oil separation efficiency. Finally, a discussion is made on improving olive oil quality via processing taking into account quality as well as production characteristics.

Χρήση χλωροπλαστικών SNPs για το προσδιορισμό της ποικιλίας προέλευσης του Ελαιολάδου

The use of chloroplastic SNPs for the identification of cultivar origin of olive oil

P. Kalaitzis, I. Manolikaki, Ch. Bazakos, T. Spanos

Five SNP regions identified from the plastome genome of olive, c.v Frantoio were employed with the purpose to develop the SNP-based PCR-RFLP methodology. In order to detect intervarietal SNPs, a screening of 5 different chloroplastic regions identified from the plastome genome of olive, c.v Frantoio (GenBank Accession Number GU931818), were performed on a set of 10 cultivars, highly prominent in Greece. Three SNPs were detected within the trnS-GCU-trnG-UCC, trnD-trnT regions and the ndhF exon, in the cultivar Lianolia Kerkyras, meaning that this specific cultivar could be differentiated among the others, by using these three SNPs, so as to be rendered a concrete chlorotype. Furthermore, PCR-RFLP capillary methodology was performed among two cultivars, Lianolia Kerkyras and Koroneiki, so as to validate the presence of SNP21. The results show that SNP21 is adequate to discriminate these two cultivars. The SNP21 was used in order to determine the varietal limit of detection using mixtures of olive leaf DNA of the two varieties Lianolia Kerkyras and Koroneiki. The mixtures analyzed by the SNP-based PCR-RFLP capillary methodology were 50/50 %, 75/25 %, 90/10 %, 95/5 % and 99/1 %. In the 50/50 % and the 75/25 % mixture, the SNP alleles of both varieties could be reliably and efficiently detected. However, this limit of detection is considered very high.

Ανάπτυξη μεθόδων ελέγχου αυθεντικότητας του ελαιολάδου με βάση ειδικούς δείκτες DNA

Development of DNA-based Olive Oil Authenticicity Tests

D.P. Kalogianni, L . Boutsika, Ch. Bazakos, T. K. Christopoulos, P. Kalaitzis

Food authenticity is of great concern for food analysis and quality assurance. In recent years there has been a growing concern among consumers over the composition of food. This has led to an increasing interest toward the development of analytical methods for testing the authenticity of food products, especially the expensive ones, in order to detect possible adulteration with similar products of lower price and nutritional value. Olive oil is one of the most vulnerable products to fraud with oils form other plants.

This project reports food authenticity tests based on specific DNA markers, such as single-nucleotide polymorphisms (SNPs). These single-base changes in DNA are particularly useful because they enable discrimination of closely related species and/or varieties. More specific, two approaches have been developed for olive cultivar identification based on the detection of several SNPs present in the plant genome. (A) A dual dipstick DNA biosensor (one strip/SNP) has been developed that provides visual identification of the olive variety by naked eye. Strongly colored gold nanoparticles are employed as reporters that enable visual detection, which is completed within few minutes. The DNA biosensor is disposable, dipstick-type using dry reagents, simple, low-cost, avoids multiple washing and incubation steps and does not require highly qualified personnel (B) A multiplex hybridization assay on spectrally distinct polystyrene microspheres has been developed for food authenticity. A multiplex allele discrimination reaction and a subsequent multiplex hybridization assay on the surface of the microspheres have been performed for several SNPs. The microspheres contain two different fluorescent dyes in different ratios generating up to 100 different sets. The detection of the hybrids is accomplished by a third fluorophore and the microspheres are analyzed by flow cytometry. The methods have been applied in real samples.

Σύγκριση της σύνθεσης των λιπαρών οξέων γάλακτος από συμβατικές και βιολογικές εκτροφές προβάτων

Comparison of fatty acid composition of milk obtained from conventional and organic dairy sheep farms

S. Maragoudakis, T. Massouras, I. Hadjigeorgiou

The aim of this study was to determine the difference in fatty acid (FA) composition of milk between organic and conventional farms. During a 2-year consecutive study, bulk sheep milk was collected, from organic (n=20) and conventional (n=16) dairy farms from three regions of Aitoloakarnania. The study was focused on FA composition with emphasis on *cis*-9, *trans*-11 CLA. Milk samples were also analyzed for chemical composition, proteins, fat, ash, mineral (Ca, Mg, Na, K) and rheological properties. Data were statistically analyzed according to factors which affected milk FA content, including farming system, region, and lactation period.

The results showed that the organic milk had a higher content of PUFA (P<0,001), MUFA (P<0,001), CLA (P<0,001) and lower of SFA (P<0,001) compared with the respective conventional milk. Significant difference was also found in Atherogenicity Index (AI) between the milk of two farming systems. (AI) was lower in the organic milk (2,72 vs 3,66). The results for the fatty acids were further investigated with a principal components analysis (PCA), showing a clear discrimination between the organic and the conventional milk samples. The protein as well as mineral Mg, Na (P<0,001), Ca and K (P<0,01) contents were much higher in organic groups than conventional one (P<0,001). The differences, especially in FA composition might be attributed to access of sheep to fresh grazing, for a longer period of time than in the conventional farming system, making the rations of greater quality. Also, some differences observed between the organic milk, attributed to the year effect, may be due to weather changes affecting milk composition through forage availability, quality and intake.

Μεθοδολογία διεξαγωγής της εθνικήςς έρευνας για την υγεία και τη διατροφή (ΠΡΟΓΡΑΜΜΑ ΥΔΡΙΑ) [1]

The Greek national nutrition and health survey "HYDRIA": Method of implementation

E. Valanou, D. Oikonomidou, A. Androulidaki, I. Gkoufa, M. Kritikou, M. Pantzalis, P. Vidalis, I. Ziara, A. Naska, A. Trichopoulou

Introduction: Nutrition and health monitoring is of prime interest in planning, implementing and evaluating public health strategies and actions.
Objective: The project HYDRIA (after HYgeia, Diet, Research In All) aims to collect highly standardized and comparable to those of other European countries data on health status, dietary intakes, prevalence of overweight and obesity and health-related lifestyle choices in a sample of the Greek resident population.
Setting: The study sample will consist of more than 4,000 individuals aged 18 years and over, representative of the Greek population based on the most recent 2011 census.
Data collection: The fieldwork procedures include the collection of:

a) dietary data, through the administration of two 24-hour dietary recalls and a non-quantitative food frequency questionnaire per participant. In addition, participants will be asked to reply to a questionnaire addressing their habits and attitudes in relation to eating out
b) information on health status, including prevalent diseases and chronic conditions as well as the participants' perception of personal health
c) data on physical activity, smoking habits and other lifestyle choices
d) measured blood pressure
e) data on measured height, weight, waist and hip circumferences
f) blood samples for the determination of blood glucose, total and HDL cholesterol

Regional Health Centers and other primary healthcare units will operate as the survey examination sites.
Conclusion: The HYDRIA study is the first Greek national nutrition and health survey involving a nationally representative population sample. The results will provide reliable and comparable information on the dietary intakes of the population in Greece and thus provide support for the planning of evidence-based public health nutrition strategies.

[1] Project Title: "HYDRIA. Program and focused action on the health and nutrition of the Greek population: development and implementation of methodology and documentation". MIS Code: 346816. Beneficiary: Hellenic Health Foundation. Sectoral operational program: Human Resource Development. Programming period: National Strategic Reference Framework (2007-2013). With the co-financing of the European Union.

3η Συνεδρία: Εφαρμογές βιοτεχνολογίας. Μεταβολισμός και βιολογική δράση Λιπιδίων

	Προεδρείο:	Β. Ωραιοπούλου Θ. Σωτηρούδης Σ. Παπανικολάου Ντ. Γαλανοπούλου
O-15	Comparative study of homogenization techniques for w/o olive oil nanoemulsions preparation	V. **Polychniatou**, C. Tzia
O-16	Lipase activity in *Nannochloropsis sp*	M.-T. G. **Savvidou**, T. G. Sotiroudis, F. N. Kolisis
O-17	Lipase catalysed reactions of fatty acids in nanodispersions based on amphiphilic block copolymers	V. **Sereti**, M. Zoumpanioti, S. Pispas, A. Xenakis
O-18	Optimization of the chemo-enzymatic epoxidation of oleic acid catalyzed by *C. antarctica* lipase immobilized in HPMC-based organogels	A. F. Zanette, I. **Zampakidi**, F. de Abreu Corrêa, M. Zoumpanioti, I. Correa Ramos Leal, R. O. Mendonça Alves de Souza, L. Cardozo Filho, A. Xenakis
O-19	*Yarrowia lipolytica*: A model microorganism used as cell factory in lipid biotechnology	S. **Papanikolaou**, G. Aggelis
O-20	Determination of PLA_2 activity in biological samples	A. Karkabounas, E.I. Kitsiouli, G. Nakos, M.E. **Lekka**
O-21	Effect of endocannabinoids on platelet activation and their hydrolysis to arachidonic acid by FAAH and MAGL	E. **Gkini**, M. Antonelou, I. Papassideri, M. Mavri-Vavayianni, A. Siafaka-Kapadai
O-22	Effect of phenolic compounds on PAF biosynthesis induced by IL-1β in U-937 cells	I. C. **Vlachogianni**, E. Fragopoulou, G. M. Stamatakis, S. Antonopoulou

Συγκριτική μελέτη των τεχνικών ομογενοποίησης για το σχηματισμό W/O νανογαλακτωμάτων με βάση το ελαιόλαδο

Comparative study of homogenization techniques for w/o olive oil nanoemulsions preparation

V. Polychniatou, C. Tzia

Emulsion droplet size is a crucial property for emulsions since affecting their stability, color, appearance, texture and rheology. Nanoemulsions contain ultrafine droplets (d < 200 nm) and thus present a number of potential advantages over conventional emulsions for the encapsulation and delivery of nonsoluble substances in foods and beverages. Nanoemulsions are characterized by high optical clarity, physical stability and increased bioavailability. The aim of this study was to evaluate the homogenization methods applied for the nanoemulsion formulation and especially to investigate the ultrasonic and high speed homogenization methods and their combination in w/o olive oil nanoemusions containing Tween 20 and water. In the emulsification formulation experiments a high speed homogenizer (CAT Unidrive 1000, CAT Scientific, Ca., USA) at 8000, 10000 and 12000 rpm and an ultrasonic generator VC 750 (Sonic & Materials) at 20 kHz at the amplitudes of 150, 225 and 300 W for 10 and 20 min were used. The nanoemulsions' properties were comparatively evaluated related to the preparation method in terms of droplet diameter, ζ-potential, emulsion stability, turbidity and color. The emulsification conditions (temperature, time, rpms, amplitude) that influence the final emulsion were studied. The results of this study concluded that stable olive oil nanoemulsions were obtained with both methods and moreover the most efficient method and conditions of emulsification were determined.

Ενζυμική δραστικότητα λιπάσης στον μικροοργανισμό *Nannochloropsis sp*

Lipase activity in *Nannochloropsis sp*

M.-T. G. Savvidou, T. G. Sotiroudis, F. N. Kolisis

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3), enzymes with significant industrial applications, are part of the family of hydrolases that act on carboxylic ester bonds. They are widely found throughout the animal and plant kingdoms, as well as in molds and bacteria. In this work we describe the detection for the first time and the partial purification of an intracellular lipase from *Nannochloropsis sp*. *Nannochloropsis* is an oleaginous eukaryotic marine alga, extensively studied in aquaculture due to its nutritional value and the ability to produce valuable chemical compounds, such as pigments and polyunsaturated fatty acids. Recently, the genomic structure of *Nannochloropsis sp.* has been analyzed.

In our work, cultures were grown in sterilized seawater enriched with f/2 medium nutrients under various illumination conditions and the effect of culture conditions on total cell biomass and chlorophyll content was recorded. Total whole cell or extracted lipase activity was measured spectrophotometrically using p-nitrophenyl laurate as substrate. Following biomass collection, cells were disrupted in a laboratory sonicator. The crude extract was concentrated with ammonium sulfate precipitation (80% saturation level) and dialyzed.

Results demonstrated that both whole cells and cell extracts express significant lipase activities. A very interesting finding was that the highest lipase activity of the partially purified extracted enzyme occurred when cultivating *Nannochloropsis sp*. without illumination, as opposed to cell culture with continuous illumination at a light intensity of 100μmol.m^{-2}.s^{1} on the surface of the flasks. The biochemical characterization of the enzyme (including thermostability, kinetic properties, etc.) is underway. The ammonium sulfate purified lipase could tolerate pH values 6.0–8.0 with maximum activity values obtained at pH 7.0.

Αντιδράσεις λιπαρών οξέων καταλυόμενες από λιπάσες που πραγματοποιούνται σε νανοδιασπορές βασισμένες σε συμπολυμερή

Lipase catalysed reactions of fatty acids in nanodispersions based on amphiphilic block copolymers

V. Sereti, M. Zoumpanioti, S. Pispas, A. Xenakis

Amphiphilic block copolymers attract increasing attention because of their wide use in various applications, such as the stabilization of oil-in-water (o/w) nanoemulsions for the encapsulation of hydrophobic molecules, such as lipophilic drugs. A more recent application of amphiphilic block copolymers could be their utilization for the stabilization of water-in-oil nanodispersions in which enzymes could be encapsulated and used for biocatalysis of lipids.

In the present work we investigated the ability of three biocompatible amphiphilic block copolymers, PEO-PCL H2, PEO-PCL H3 and PEO-PCL H4 to act as surfactants for the stabilization of water-in-oil nanodispersions, in which enzymatically catalyzed reactions can take place. The copolymers used consist of hydrophilic poly(ethylene oxide) and hydrophobic poly(ε-caprolactone) and differ on the hydrophilic/hydrophobic block ratio. To begin with, phase diagrams for the three block copolymers in mixtures of chloroform/isopropanol/water were constructed. From these phase diagrams it was concluded that the systems PEO-PCL H3:chloroform/isopropanol/water and PEO-PCL H4:chloroform/isopropanol/water were capable of solubilizing an adequate amount of aqueous phase. The phase inversion temperature of the studied systems was also determined. The water-in-oil nanodispersions were then characterized by Dynamic and Static Light Scattering.

Subsequently the systems previously mentioned were used to encapsulate *R. miehei* lipase and examine whether its catalytic activity is maintained as in relative w/o microemulsions. It was proven that the model esterification reaction of 1-propanol with lauric acid (a fatty acid) was successfully catalyzed by *R. miehei* lipase in these w/o nanodispersions. Finally the effect of temperature on the catalytic behavior of *R. miehei* lipase encapsulated in the previously described water-in-oil nanodispersions was investigated. It was observed that the dependence of the initial rate of the esterification reaction differs between the water-in-oil nanodispersions stabilized by PEO-PCL H3 and the water-in-oil nanodispersions stabilized by PEO-PCL H4.

Optimization of the chemo-enzymatic epoxidation of oleic acid catalyzed by *C. antarctica* lipase immobilized in HPMC-based organogels

A. F. Zanette, I. Zampakidi, F. de Abreu Corrêa, M. Zoumpanioti, I. Correa Ramos Leal, R. O. Mendonça Alves de Souza, L. Cardozo Filho, A. Xenakis

Epoxides are intermediate compounds for the production of polymers, adhesives, resins and other materials. This versatility is associated with the high reactivity of their oxirane ring. The chemo-enzymatic epoxidation reaction constitutes a selective and environmentally benign alternative to the traditional Prilezhaev epoxidation process, currently used in industry to convert unsaturated compounds to the corresponding epoxides.

Organogels-containing lipases are systems that can be successfully used as media for hosting various biocatalytic reactions. Enzymes encapsulated in these systems can retain their catalytic activity and have already been used for the catalysis of the esterification of lipids as well as phenolic acids.

In the present work, the use of C. antarctica lipase immobilized in hydroxyl-propyl-methyl cellulose (HPMC)-based organogels, as media for the catalysis of oleic acid epoxidation with H_2O_2, has been tested. The optimization of several reaction conditions such as temperature, concentration of substrates and the enzyme content in the organogel, have been examined. For this purpose an experimental design (central composite rotatable design – CCRD) was applied and results were compared with experiments conducted with PSCI Amano lipase from Burkholderia cepacia immobilized on ceramic. Based on the optimum conditions as indicated by CCRD, the kinetics of the reaction were studied.

Yarrowia lipolytica: Ένας μικροοργανισμός μοντέλο χρησιμοποιούμενος ως κυτταρικό εργοστάσιο στη βιοτεχνολογία των λιπαρών υλών

Yarrowia lipolytica: A model microorganism used as cell factory in lipid biotechnology

S. Papanikolaou, G. Aggelis

This review presents a survey of studies related with the production of microbial metabolic compounds during the cultivation of the yeast *Yarrowia lipolytica* on a plethora of "oleochemical-type" residues utilized as substrates; the potentiality of growth on stearin (a low-cost derivative of tallow composed of saturated free-fatty acids) was assessed, and significant biomass production was obtained (yield of more than 1 g of biomass produced per g of fat consumed), accompanied by notable intra-cellular lipid accumulation and production of lipase. Due to the fact that a spontaneous rise in the concentration of cellular stearic acid was observed, hydrolyzed oleic rapeseed oil or biodiesel-derived waste glycerol was used as co-substrate in order to increase the concentration of cellular oleic acid and, therefore, to produce substitutes of exotic fats. Indeed, correct use of the above-mentioned low-cost products, resulted in the intra-cellular synthesis of a lipid presenting composition similarities with the high-value cocoa-butter. In the cultures with crude glycerol as co-substrate, non-negligible quantities of citric acid were synthesized. Use of crude glycerol as the sole substrate used at high initial concentrations (e.g. up to 165 g/L), thus, resulted in the production of ~63 g/L of citric acid. The fact that the microorganism produced citrate in nitrogen-limited media composed of glycerol or similarly metabolized compounds (glucose) resulted in the concept to use (potentially glucose-enriched) olive-mill wastewaters as citrate-production media. Indeed, in these types of media, the microorganism produced non-negligible citrate and biomass quantities, reducing simultaneously the color and the phenolic content of the effluents.

Προσδιορισμός ενεργότητας φωσφολιπάσης A_2 σε βιολογικά δείγματα

Determination of PLA_2 activity in biological samples

A. Karkabounas, E. I. Kitsiouli, G. Nakos, M.E. Lekka

Phospholipases A_2 (PLA_2s) are a family of enzymes that catalyze the hydrolysis of the *sn-2* ester bond of phospholipids, liberating free fatty acids and lyso-phospholipids. They are considered to be inflammation markers and their determination could be crucial. In this framework, the purpose of the present study was the development and evaluation of various assays for the determination of total PLA_2 activity in biological samples.

Firstly, we have reported an integrated study on a real-time fluorimetric assay for the determination of PLA_2 total activity and PAF-AH, using C_{12}-NBD-PC and C_6-NBD-PC as substrates, respectively. The liberation of the relevant C_{12}- or C_6-NBD-Fatty Acid was measured from the fluorescence increase of the reaction mixture due to PLA_2 activity. The assay presented limitations related to high protein content, color or turbidity of the samples.

In order to overcome such limitations we combined a real-time fluorimetric assay with an additional HPLC step for the separation of the fluorescent substrates from their relevant fatty acid products. The assay was time-consuming and difficult to be automated.

Towards this direction, C_{12}- and C_6-NBD-PC substrates were immobilized by entrapment in a polymer matrix and a fluorimetric method for the determination of PLA_2 total activity in biological samples, using the immobilized substrates, was developed and evaluated. This assay overcomes the above limitations and it could be automated in order to be used for the analysis of several samples simultaneously. For this innovative study a patent has been granted from the Greek Industrial Property Organization.

Επίδραση των ενδοκανναβινοειδών στην ενεργοποίηση των αιμοπεταλίων και η υδρόλυσή τους σε αραχιδονικό οξύ από την αμιδοϋδρολάση των λιπαρών οξέων και τη λιπάση των μονοακυλογλυκερολών

Effect of endocannabinoids on platelet activation and their hydrolysis to arachidonic acid by FAAH and MAGL

E. Gkini, M. Antonelou, I. Papassideri, M. Mavri-Vavayianni, A. Siafaka-Kapadai

Endocannabinoids are lipid signaling molecules involved in a variety of physiological and pathological conditions. The two main endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (N-arachidonoylethanolamine, AEA) are synthesized by neurons and other cells, including platelets, in a stimulus-dependent manner. Following their transport into cells they are inactivated by enzymatic degradation. AEA is inactivated by fatty acid amide hydrolase (FAAH), whereas 2-AG is inactivated by the action of both FAAH and monoacylglycerol lipase (MAGL). In this study, the degradation process of 2-AG by FAAH and MAGL and the characterization and identification of the enzymes in rabbit platelets using immunoblot analysis and immunocytochemistry is reported. We suggest that platelets are activated by the endocannabinoid 2-AG via its hydrolysis to arachidonic acid. Preincubation of platelets with FAAH and MAGL inhibitors affected the aggregation induced by 2-AG and other platelet agonists (PAF, thrombin). Regarding human platelets, reports are controversial; the activation of platelets by endocannabinoids through the activation of cannabinoid or “platelet type” receptors has been reported while other reports, including results from our group suggest that platelets are activated, at least in part, by the produced arachidonic acid. Finally, our finding regarding the presence (and alterations) of PPARgamma in platelets, suggest a role for this nuclear factor in platelet activation induced by endocannabinoids. The functional role of endocannabinoids in platelets is not clear. It appears though that these lipids play a major role in the arachidonic acid homeostasis and the consequent effects on platelet activation and suggest that platelets contribute to the inactivation of the endocannabinoids.

Η επίδραση φαινολικών ενώσεων στην επαγόμενη από IL-1β σύνθεση PAF στη κυτταρική σειρά U-937

Effect of phenolic compounds on PAF biosynthesis induced by IL-1β in U-937 cells

I. C. Vlachogianni, E. Fragopoulou, G. M. Stamatakis, S. Antonopoulou

Platelet activating factor is a potent lipid mediator, which is produced by many cell types including monocytes. Interleukin 1beta (IL-1β) induces PAF synthesis in U-937 via the augmentation of its biosynthetic enzymes. The purpose of this research is to study the effect of phenolic compounds on PAF production in U-937 after IL-1β stimulation.

U-937 stimulated by IL-1β (2.5ng/mL) in two different time points in presence or absence of resveratrol and tyrosol (5-400μM). In all conditions, PAF levels have been measured as well as the specific activities of acetyl-CoA:lyso- PAF acetyltransferase (lyso-PAF AT), and DTT-insensitive CDP-choline 1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase (PAF-CPT) in cells homogenates.

IL-1β induces an elevation of intracellular PAF levels at 0.5h and 3h (2-fold) and increased lyso-PAF-AT activity (2-fold) at 3 hours and PAF-CPT activity (1.5 fold) at 0.5 hours. Tyrosol inhibited the IL-1β induced actions with IC_{50} value for intracellular PAF and lyso-PAF-AT at the order of 50 μM (3 hours) and for PAF-CPT at the order of 250 μM (0.5 hour).

Resveratrol exhibited similar inhibitory action with tyrosol, with IC_{50} value for intracellular PAF and lyso-PAF-AT at the order of 150 μM (3 hours) and for PAF-CPT at the order of 300 μM (0.5 hour).

The anti-inflammatory effect of resveratrol and tyrosol seems to be partly achieved by reduction of lyso-PAF-AT activity, which is consistent with the reduction in the intracellular PAF levels. It should be noticed that tyrosol seems to be more potent inhibitor than resveratrol. Both phenolic compounds also inhibited PAF-CPT activity but in higher concentrations. These actions may explain phenolic compounds antiatherogenic properties.

Β. Περιλήψεις Πόστερς

1	Preparation and characterization of Chios mastic gum fractions before and after encapsulation in liposomes by three different methods	O. Gortzi, V. Athanasiadis, S. Lalas, J. Tsaknis
2	Use of olive oil for frying is associated with higher likelihood of acute coronary syndrome and ischemic stroke non-fatal events: a case/case control study	C.-M. Kastorini, H. J. Milionis, Z. Konidari, E. Ntziou, S. Bitsi, V. Euthimiou, V. Nikolaou, K. N. Vemmos, J. A. Goudevenos, D. B. Panagiotakos
3	Microencapsulation of limonene using Acacia gums of different chemical composition	T. Koupantsis, C. Malhiac, F. Renou, A. Paraskevopoulou
4	Use of polar compounds sensor for frying process monitoring	C. Chranioti, S. Chanioti, V. Polychniatou, P. Sfakianakis, D. Tsimogiannis, V. Giannou, C. Tzia
5	Microbial conversions of biodiesel-derived waste glycerol into added-value compounds with the use of yeast and fungal strains	A. Chatzifragkou, M. Mavrou, A. Koutinas, G. Aggelis, S. Papanikoloaou
6	Effect of exogenous 1,3-propanediol on cellular lipid profile of *Clostridium butyricum* during growth in batch and continuous cultures	A. Chatzifragkou, G. Aggelis, M. Komaitis, A. Koutinas, S. Papanikolaou
7	Headspace Solid Phase Microextraction procedure for mastic gum “Green” chemical analysis	H. Damianakos, K. Graikou, J. Tsaknis, I. Chinou
8	Lipids synthesized by strains of the medicinal edible fungi *Volvariella volvacea* during their cultivation in liquid growth medium	P. Diamantopoulou, A. Philippoussis, S. Papanikoloaou, M. Komaitis, G. Aggelis
9	Freeze Drying of fennel plants: Use of biopolymers as surface barriers	C. Gardeli, V. Evageliou, C. Poulos, S. Yanniotis, M. Komaitis

10	Study of the possible phosphatidylinositol 5-phosphate presence in *T. thermophila* cells	D. Deli, G. Katsipis, S. Thiveos, S. Dika, G. Leontaritis, D. Galanopoulou
11	Effect of storage conditions in the content of Alkyl esters in Greek Extra Virgin Olive Oils	A. Gali, G. Exarchos, Ch. Kapiniaris, P. Zoumpoulakis, V. Gergis
12	The fatty acid composition of donkey and camel milk : a review	A. Georgala
13	Chemical characteristics of traditional Greek cheeses of the Aegean Sea islands	A. Georgala
14	Production of microbial oil from flour-based industrial waste streams	S. Tsakona, N. Kopsahelis, A. Chatzifragkou, S. Papanikolaou, A.A. Koutinas
15	Fatty acids profile of milk from Greek sheep breeds	P. Koutsouli, V.J. Sinanoglou, G. Sotiropoulou, A. Klavdianos, K. Sotirakoglou, I. Bizelis
16	Oil bodies recovery by applying ultrafiltration and their exploitation for the preparation of composite sodium caseinate-based edible films	A. Matsakidou, M. Tsimidou, V. Kioseoglou
17	Study of factors affecting fungal growth and the biosynthesis of their carcinogenic metabolites	D. M. Meimaroglou, F. Flouri, D. Galanopoulou, P. Markaki
18	Antioxidant activity of three natural phenoliic antioxidants in various vegetable oils: a comparative study	E. Vagena, I. Papadaki, D. Tsimogiannis, V. Oreopoulou
19	HPLC-DAD and GC-MS analysis of phenolic compounds in extra virgin olive oils	Ch. Proestos, M. Komaitis, P. Zoumpoulakis, V. Sinanoglou
20	Antioxidant capacity of plant extracts and essential oils by the Rancimat test. Determination of lipid oxidation and stability	Ch. Proestos, M. Komaitis, P. Zoumpoulakis, V. Sinanoglou
21	Bio-ethanol production during growth of the	D. Sarris, L. Matsakas, A.

	yeast *Saccharomyces cerevisiae* MAK 1 on mixtures of molasses and olive mill wastewaters under non-sterile conditions	A. Koutinas, M. Komaitis, S. Papanikolaou
22	Lipid profile study of the edible fungus *Laetiporus Sulphureus*	V.J. Sinanoglou, P. Zoumpoulakis, J. Petrovic, J. Glamoclija, M. Sokovic, Ch. Proestos, G. Heropoulos
23	Quality changes of semi-preserved *Mugil cephalus* ovaries (avgotaracho), during storage at 3.0 ± 1.0 ºC	V.J. Sinanoglou, M. Voulgarelis, P. Androutsaki, I.F. Strati, V. Oreopoulou, V.P. Lougovois
24	A study of olive kernel oil extraction and bioactive compounds recovery using mixed-polarity solvents	S. Chanioti, C. Tzia
25	Secretion of monoacylglycerol lipase and fatty acid amide hydrolase in *Tetrahymena thermophilα*	A. Stamogiannos, E. Gkini, D. Galanopoulou, A. Velentzas, M. Antonelou, I. Papassideri, A. Siafaka-Kapadai
26	Effect of extraction system and conditions, malaxation time and temperature, on the quality characteristics of virgin olive oil	K. Kotsiou, K. Gennatos, M. Tasioula-Margari
27	Antiproliferative action of pumpkin seed lipid extracts on PC-3 prostate cancer cells	R. Tenta, M. Xanthopoulou, M. Tsoukala, H. Pratsinis, D. Kletsas, S. Antonopoulou, T. Nomikos
28	Antiproliferative effects of red and white wine extracts in PC-3 prostate cancer cells	R. Tenta, M. Xanthopoulou, M. Tsoukala, H. Pratsinis, D. Kletsas, S. Antonopoulou, E. Fragopoulou
29	Antiplatelet effect and phytosterol content of nuts' lipid extracts	I. C. Vlachogianni, N. Kalogeropoulos, S.

		Antonopoulou, C. A. Demopoulos, T. Nomikos
30	Lipid production during growth of the yeast *Cryptococcus curvatus* on lactose-enriched olive mill waste-waters	E. Xenopoulos, A. Chatzifragkou, A. A. Koutinas, S. Papanikolaou
31	Fatty acid composition and sterol content of Greek traditional Milk-cereal foods	C. Chatzi, E. Zoidou, T. Massouras
32	Variation in fatty acid composition of ewe‘s milk during dietary supplementation with hesperidin	K. Moschou, T. Massouras, E. Zoidou, S. Deligiorgis, J. Bizelis
33	Study of olive oil antioxidants & in vitro antioxidant activity, during ripening process of olives var. “Koroneiki”	A. Artemiou, N. Kalogeropoulos, A. Kaliora, A. Chiou
34	Effect of size on the sensory characteristics and fillet composition of farmed meagre fish (Argyrosomus regius)	J. Giogios, K. Grigorakis, N. Kalogeropoulos
35	Lipid microemulsions and their potential as delivery systems for bioactive compounds	A. Kalaitzaki, V. Papadimitriou, A. Xenakis
36	Interfacial properties and their relation with the technology and quality in dietary oils and fats	E.P. Kalogianni
37	Capillary rise in porous media to set rejection criteria for reused fried oils	J. Lioumpas, A. Zamanis, Th. Karapantsios
38	Use of liquefied dry sweet sorghum stalks for the production of lipids by Lipomyces starkeyi CBS 1807 cells	L. Matsakas, A. A. Sterioti, A. Spanopoulos, P. Christakopoulos
39	Monoolein production under High-Pressure Vapor-Liquid Equilibrium	F. Zanette, L. Ferreira Pinto, I. Correa Ramos Leal, R. O. Mendonça Alves de Souza, L. Cardozo Filho
40	Effects of red and white wine extracts on PAF biosynthetic enzymes	M. N. Xanthopoulou, D. Asimakopoulos, S. Antonopoulou, E. Fragopoulou

Παρασκευή και χαρακτηρισμός των εκχυλισμάτων της μαστίχας Χίου πριν και μετά τον εγκλωβισμό τους σε λιποσώματα με τρεις διαφορετικές μεθόδους

Preparation and characterization of Chios mastic gum fractions before and after encapsulation in liposomes by three different methods

O. Gortzi, V. Athanasiadis, S. Lalas, J. Tsaknis

Chios mastic gum, the resin obtained as an exudate from the trunk and branches of *Pistacia lentiscus* L var. *chia,* has found extensive use in pharmaceutical products and as a nutritional supplement. The oral absorption of crude resin (contained a high percentage of an insoluble and sticky polymer of poly-β-myrcene) is poor due to its low water-solubility and reduces the bioavailability of the contained active compounds.

A total mastic extract without polymer (TMEWP) was prepared after removal of the contained insoluble polymer in order to ameliorate solubility and enhance in vivo activity. To further characterize potential active mastic constituents, the TMEWP was separated into an acidic and a neutral fraction. To overcome the drawbacks of ME, the selection of a suitable carrier is very necessary and crucial. Three different methods of preparation, thin film evaporation (TFE), solid lipid nanoparticles (SLN), and ethanol injection (EI) used for the preparation of liposomes consisting of phosphatidylcholine (PC) and cholesterol (CH). The effect of PC:CH molar ratio on the percentage of mastic extract encapsulated was investigated. Mastic gum extracts components-liposomes interaction was studied using Fourier transform infrared (FT-IR) spectroscopy and differential scanning calorimetry (DSC). The effects of different preparation methods on the physicochemical properties of liposomes were evaluated by means of surface morphology by field emission scanning electron microscopy, zeta potential and size distribution using a Zetasizer and particle size analyser, respectively.

The study has been co-funded by 75% from E.E. and 25% from the Greek Government under the framework of the Education and Initial Vocational Training Program-Archimedes III.

Use of olive oil for frying is associated with higher likelihood of acute coronary syndrome and ischemic stroke non-fatal events: a case/case control study

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During 2009-2010, 1000 participants were enrolled; 250 were consecutive patients with a first ACS, 250 were consecutive patients with a first ischemic stroke and 500 population-based, control subjects, one-for-one matched to the patients by age and sex. Socio-demographic, clinical and other lifestyle characteristics were measured. Dietary patterns were evaluated using a validated semi-quantitative food frequency questionnaire.

Results: After adjusting for various potential confounding factors (age, sex, physical activity, body mass index, family history of cardiovascular disease, smoking, hypertension, hypercholesterolemia, diabetes mellitus and MedDietScore), use of olive oil for frying more than one time per week, compared with less than weekly, was associated with 72% higher likelihood of ACS (95%CI: 1.02-2.91) and more than double likelihood (OR: 2.29, 95%CI: 1.28-4.10) of ischemic stroke.

Μικροενθυλάκωση λεμονενίου με χρήση δειγμάτων αραβικού κόμμεος διαφορετικής χημικής σύστασης

Microencapsulation of limonene using Acacia gums of different chemical composition

T. Koupantsis, C. Malhiac, F. Renou, A. Paraskevopoulou

Microencapsulation proved to be a convenient strategy to protect hydrophobic aroma compounds, as well as functional components, from factors that may cause deterioration. As wall materials (encapsulating agents) have been used various biopolymers with emulsifying and stabilizing activity. *Acacia* gum has been the encapsulating agent of choice for many years because it is an excellent emulsifier, has a bland flavour and provides very good volatiles retention during the drying process. It is described as a complex acidic branched polysaccharide, obtained as an exudate from *Acacia* trees, containing about 2% protein. Its emulsifying capacity is attributed to its ArabinoGalactanProtein fraction (AGP) where most of the protein is located. As most natural products, *Acacia* gum is subject to chemical variability that sharply affects its functional properties.

In this study, the effect of *Acacia* gum chemical composition on microencapsulation of limonene has been explored. For this reason, three *Acacia* gums, differing in their AGP content, were used, i.e. low (GA1), medium (GA2) and high (GA3), and their physicochemical characteristics were first examined. The selection of limonene as material being encapsulated was based on the fact that it is a naturally occurring hydrocarbon, practically insoluble in water, commonly found in essential oils.

The microencapsulation of limonene was achieved by emulsification and subsequent water removal by applying the freeze drying technique. The initial emulsions were firstly compared in terms of their droplet size, all emulsions displayed surface-weighted mean ($d_{3,2}$) droplet sizes that fell within 1.061–1.122 μm, while the formation of limonene microcapsules was confirmed by Scanning Electron Microscope observation. The microencapsulation process was monitored by microencapsulation efficiency, microencapsulation loading capacity and microencapsulation yield. According to our results, the microencapsulation efficiency was independent of *Acacia* gum used. All three Acacia gums showed only slight variations in their encapsulation parameters (p>0.05) as well as their morphology.

Χρήση αισθητήρα πολικών συστατικών για τον έλεγχο της διαδικασίας τηγανίσματος

Use of polar compounds sensor for frying process monitoring

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Frying is an important process which is used to alter the eating quality of foods by means of hot oil or fat, while simultaneously offering a preservative effect due to microorganisms destruction and water activity reduction of food. Frying is widely used for the preparation of various fried foods providing them desirable colour, texture and flavour. These positive attributes result from the physical-chemical reactions that take place during frying providing a characteristic deep-fried flavour which makes fried foods appealing for consumers and especially for young people.

However, frying can also negatively affect both frying oils and fried food. Deep frying, which occurs at high temperatures (>180°C) frequently involves fat deterioration due to extended chemical (oxidation, polymerisation, hydrolysis, colour formation) and physical (odour/flavour development) reactions that produce degradation compounds (such as trans fatty acids, highly oxidised or polymerised constituents of fatty acids and acrylamide) that affect the functional, sensory and nutritional qualities of oils and fried food even more potentially harmful for human health.

As far as food safety is concerned, the implementation of HACCP and the control of the critical for the safety stages (CCPs) are mandatory in food processes under current legislation. Frying is considered a critical stage both for the quality (CP) and safety (CCP), while the change of fat characteristics should be monitored either by chemical tests or continuously by using sensors. The extent of fat deterioration may be evaluated by various quality parameters of fat/oil (e.g. anisidine value, total polar materials). However, the continuous monitoring of fat quality can also be achieved through rapid analyses using test kits or appropriate sensors based on dielectric constant measurements. In the present work the on-line monitoring of fat deterioration by suitable sensors is proposed based on experimental data that should assure safe frying processes.

Μικροβιακές μετατροπές της απόβλητης από τη διεργασία παραγωγής βιοντήζελ γλυκερόλης σε προϊόντα προστιθέμενης αξίας με τη χρήση στελεχών μυκήτων και ζυμών

Microbial conversions of biodiesel-derived waste glycerol into added-value compounds with the use of yeast and fungal strains

A. Chatzifragkou, M. Mavrou, A. Koutinas, G. Aggelis, S. Papanikoloaou

In the current submission, *Yarrowia lipolytica* LFMB 19, *Pichia membranifaciens* LFMB 8 and *Thamnidium elegans* CCF-1465 converted raw glycerol, waste deriving from biodiesel production, into value-added metabolic products. Cultures were performed in media favoring the production of organic acids and/or lipid (single cell oil-SCO) in the case of *Y. lipolytica* and *T. elegans*, or microbial mass (single cell biomass-SCB) formation in the case of *P. membranifaciens* under increasing initial glycerol concentrations, namely 30, 60 and 90 g/L. *Y. lipolytica* produced significant amounts of acetic acid (29.2 g/L), as well as mannitol (19.4 g/L) in elevated glycerol concentrations, while citric acid concentration did not exceed 9.4 g/L. *P. membranifaciens* grew well on glycerol, reaching 28.4 g/L of SCB at initial glycerol concentration of 90 g/L. Raw glycerol was an excellent substrate for SCO production by *T. elegans*, yielding 11.6 g/L of oil, with 71% (wt/wt) of SCO accumulated in dry biomass produced, containing 371 mg/L of GLA. Lipid yield of glycerol consumed presented the appreciable values of 0.20-0.22 g/g. Further analysis of the lipids produced for the microorganisms tested, revealed that the unsaturation index of total lipid produced for both *Y. lipolytica* and *P. membranifaciens* increased with increment of glycerol concentration into the culture medium. Finally, fractionation of *T. elegans* lipids indicated that the neutral fraction presented almost similar fatty acid composition with total lipids, while the fraction of phospholipids was noticeably more enriched in polyunsaturated fatty acids and specifically GLA.

Επίδραση της εξωγενούς 1,3-προπανοδιόλης στο προφίλ των κυτταρικών λιπιδίων του *Clostridium butyricum* κατά την αύξηση του σε ασυνεχή και συνεχή συστήματα καλλιέργειας

Effect of exogenous 1,3-propanediol on cellular lipid profile of *Clostridium butyricum* during growth in batch and continuous cultures

A. Chatzifragkou, G. Aggelis, M. Komaitis, A. Koutinas, S. Papanikolaou

During 1,3-propanediol (PDO) fermentation, high diol concentrations in the culture medium could evoke a stressful environment to growing bacterial population, which might induce alterations in the fatty acid composition of their cellular lipids. On these grounds, aim of the present study was to identify the effect of exogenous PDO on the cellular lipid profile of a natural PDO-producer bacterial strain, *Clostridium butyricum* VPI 1718. Batch and continuous trials were carried out during which increasing amounts of PDO were introduced into the culture during exponential growth phase or, alternatively, at steady-state. Subsequently, cellular lipid composition was monitored in order to evaluate possible alterations in the presence of exogenous PDO. Indeed, significant variations were observed during batch trials of the strain, since the addition of PDO during batch cultures caused an increment in the percentage of unsaturated fatty acids. In particular, palmitoleic, oleic and vaccenic acids were enhanced, contrary to the concentrations of palmitic and myristic acids. Moreover, the same trend was observed in the presence of exogenous PDO at chemostat steady-states. This was further highlighted by the alteration of the saturated-to-unsaturated ratio, which was decreased from 2.29 to 1.84 after PDO addition. However, it should be stated that during transitory phase and after the culture entered again into steady-state mode, the percentages of fatty acids were reverted to levels resembling to those found at initial steady-state mode, prior to any PDO addition. This observation could be attributed to the fact that continuous cultivation system offers the advantage of gradual removal of the externally added PDO, a fact that could account for the alteration of fatty acid composition between transitory phases and steady-states.

Χημική ανάλυση αερίων υπερκείμενου χώρου σε μαστίχα

Headspace Solid Phase Microextraction procedure for mastic gum "Green" chemical analysis

H. Damianakos, K. Graikou, J. Tsaknis, I. Chinou

In the framework of a project Archimidis III re-evaluating the antimicrobial activity of chios mastic gum fractions before and after encapsulation in liposomes in order to prolong the shelf life and enhance the biological activities and sensory characteristics of milk products, we present here mastic gum HSPM chemical analysis, which to our knowledge is presented, on crude natural product, for first time.

Mastic is a well-known natural resin from the trunk and branches, of *Pistacia lentiscus* var. *chia* (Anacardiaceae), which is grown as endemic only in the Greek island of Chios. It has been used in traditional Greek medicine for various gastrointestinal disorders (gastralgia, dyspepsia, peptic ulcer, etc) since antiquity. The plant has been mentioned by Hippocrates, Dioscorides, Theophrastos, and Galenos recommending its healing properties.

Recently, in several studies, mastic gum has shown very interesting antimicrobial profile against a panel of human pathogenic fungi and bacteria among which *Helicobacter pylori*.

The aim of this research was to develop a reliable analytical method based on Headspace–Solid Phase Microextraction (HS-SPME) and Gas Chromatography–Mass Spectrometry (GC-MS) in order to detect volatile components of the natural crude mastic gum. Through this analysis α-pinene (25.6%), verbenone (14.0%), β-cymene and verbenene appeared as the most abundant constituents, representing 58% of the total, among the 27 identified volatile components of the mastic.

This technique (HS-SPME) combines sampling free from organic solvents, applying to complex matrices, while it is economic, sufficiently fast and as a "green" technique friendly to the environment.

Σύνθεση λιπιδίων από στελέχη του φαρμακευτικού εδώδιμου μύκητα *Volvariella volvacea* κατά την καλλιέργειά τους σε υγρό θρεπτικό μέσο

Lipids synthesized by strains of the medicinal edible fungi *Volvariella volvacea* during their cultivation in liquid growth medium

P. Diamantopoulou, A. Philippoussis, S. Papanikoloaou, M. Komaitis, G. Aggelis

Four *Volvariella volvacea* strains (AMRL 188, 190, 191 and 192) were studied in relation with their ability to synthesize lipids in static flasks with glucose-based medium for 24 days. For all strains, biomass production was significant (13-15 g/l), whereas total intra-cellular lipids in dry weight ($Y_{L/X}$, %, w/w) were within the range of 3-12%. Maximum $Y_{L/X}$ quantity (12% w/w) was obtained at the beginning of the growing phase (8th day) and regardless of the mushroom strain, $Y_{L/X}$ declined with fermentation time. Subjecting strain *V. volvacea* 190 to agitation (120 rpm) resulted in almost two-fold increase of $Y_{L/X}$ (14%, w/w) compared to non-agitation (8%, w/w) at the beginning of the fermentation, showing also a gradual decrease through the fermentation process. At the end of the cultivation period, neutral lipids (NL) constituted the major part (up to 64%, w/w) of *V. volvacea* lipids, followed by glycolipids+sphingolipids (G+S) whereas phospholipids (P) were produced in small quantities (up to 5.5%, w/w). Nevertheless, in both static and agitated cultures of *V. volvacea* 190, the NL fraction constituted the major part of lipid at day 8 but it decreased as fermentation proceeded, whereas the G+S fraction increased. Total fatty acid (FA) analysis of lipids showed that linoleic acid ($^{\Delta 9,12}$C18:2) was predominant (up to 66%, w/w), while in the second position but in significant lower concentrations was found the palmitate (C16:0) (up to 23%, w/w). Moreover, non-negligible quantities of saturated low- or high-aliphatic chain FAs (C8:0, C10:0, C12:0, C14:0 and C20:0) accounting to 20-25% (w/w) of total lipids were detected. Additionally, in agitated conditions *V. volvacea* 190 produced more unsaturated FAs compared with those from the non-agitated ones. NL and G+S fractions were more rich in unsaturated FAs (particularly $^{\Delta 9,12}$C18:2) as compared to total lipids, whereas the P was the most saturated fraction of all.

Λυοφιλίωση μάραθου: Χρήση βιοπολυμερών ως συστήματα επιφανειακού φραγμού

Freeze Drying of fennel plants: Use of biopolymers as surface barriers

C. Gardeli, V. Evageliou, C. Poulos, S. Yanniotis, M. Komaitis

Fennel is a well known aromatic plant as well as a well-recognised medicinal plant because of its essential oil. Fennel's essential oil is stimulant, aromatic and carminative. Its characteristic is the presence of *trans*-anethole and isoanethole.

Aromatic plants are usually subjected to drying in order to drastically reduce moisture content and reduce or completely inactivate physiological, microbial, and enzymatic degradation. However, drying is accompanied by changes in the physical properties as well as the loss or deterioration of aroma compounds and other constituents possessing antioxidant activity. Freeze drying is a process with minimum loss of flavour and aroma, negligible shrinkage, loss of valuable components etc.

The present work was an attempt to minimize the losses of the two main volatile compounds of fennel's essential oil (i.e. isoanethole and *trans*-anethole) during freeze drying of the fennel plant using biopolymer solutions and gels as surface barriers. Biopolymer solutions consisted of 20 and 40 %w/w starch and gels of 2.5, 5 and 10 %w/w gelatine.

The essential oil was obtained by subjecting a known quantity of freeze dried plant material (in the presence or absence of biopolymers) to hydrodistillation in a Clevenger apparatus. Then, it was quantitatively analysed with GC-FID and the concentrations of *trans*-anethole and isoanethole were calculated.

Results showed that gelatine gels and starch solutions were effective as surface barriers in the retention of volatile compounds thus acting as a second matrix. Both led to reduction of aroma compounds losses but their concentration, type and state seemed to have no effect on this behaviour.

Μελέτη της πιθανής παρουσίας 5-φωσφορικής φωσφατιδυλοϊνοσιτόλης σε κύτταρα *T. thermophila*

Study of the possible phosphatidylinositol 5-phosphate presence in *T. thermophila* cells

D. Deli, G. Katsipis, S. Thiveos, S. Dika, G. Leontaritis, D. Galanopoulou

Phosphoinositides (PIs), a family of seven membrane phospholipids derived from phosphatidylinositol (PtdIns) by reversible phosphorylation of their headgroup, have long been known to have important regulatory roles in cell physiology: signalling for PtdIns4,5P_2 and for 3-PIs, regulation of membrane traffic, regulation of cytoskeletal and nuclear events. Furthermore, each PI has a unique subcellular distribution and, therefore, PIs define organelle identity.

PtdIns5P, the least characterized PI, appears to be a new player in the regulation of cell physiology. However, PtdIns-specific 5-kinases have not been identified in eukaryotic cells; instead, phosphatases seem to play an important role in PtdIns5P production. This implies for both PtdIns4,5P_2 4-phosphatases and PtdIns3,5P_2 3-phosphatases of myotubularin (MTM) family.

The unicellular eukaryotic organism *Tetrahymena* is an established model in lipid and membrane and, more recently, in PI research. PtdIns, PtdIns3P, PtdIns4P, PtdIns3,5P_2, PtdIns4,5P_2 are present in both the a-micronucleated *T. pyriformis* and *T. thermophila* and there is evidence for their involvement in lysosomal enzyme secretion, phagocytosis and chemotaxis. *Tetrahymena* cells contain also several isoforms of the signalling PtdIns4,5P_2-phospholipase C and an expanded family of PI kinases, one of which (TtPIKfyve/TtPIPK3) could be responsible for PtdIns5P production. In addition, analysis of *T. thermophila* genome revealed the presence of two MTM-type phosphatases that could also serve PtdIns5P synthesis.

During the course of the analysis of total deacylated *T. thermophila* PIs by HPLC, we have identified a peak with Rt corresponding to GroPtdIns5P. Based on the above information, we now focus on kinase and phosphatase inhibition for PtdIns5P identification in *Tetrahymena* cells.

Επίδραση συνθηκών αποθήκευσης στη σύσταση και συγκέντρωση των αλκυλεστέρων σε Ελληνικά Εξαιρετικά Παρθένα Ελαιόλαδα

Effect of storage conditions in the content of Alkyl esters in Greek Extra Virgin Olive Oils

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Fatty acid alkyl esters (FAAE), namely fatty acids methyl esters (FAME) and fatty acids ethyl esters (FAEE), are useful indicators for detecting the quality of extra virgin oils as their presence is related to harvesting and manufacturing processes.

In 2011, a legal limit of 75 mg/kg for the sum of FAME and FAEE, and 150 mg/kg for the sum of FAME and FAEE, if the ratio of FAEE/FAME is below 1,5, was regulated. Recently, a new limit of 30 mg/kg for FAEE is proposed by IOC.

In this study, 23 Greek extra virgin oils, representative of different origin and varieties, PDO and PGI, unfiltered and of biological farming, were selected , and storage trials were carried out to verify that FAME and FAEE do not change under different storage conditions.

The samples were analyzed for FAAE by the IOC method for alkyl esters and waxes (COI/T.20/Doc. No 28), before and after being stored for a 4 month period at room temperature, in the dark and in the presence of light. Six of the samples were exposed to direct sunlight for 3 days to study the effect of sunlight on FAME and FAEE and six samples were also stored in dark at a steady temperature of 35^{o}C to study the effect of temperature in the content of FAAE.

To analyze the data, *one way ANOVA* was used, and it was statistically verified that there are no significant changes in the concentration of FAME and FAEE during storage of the samples in dark and light at room temperature.

Η σύσταση σε λιπαρά οξέα του λίπους του γάλακτος της γαϊδούρας και της καμήλας : βιβλιογραφική ανασκόπηση

The fatty acid composition of donkey and camel milk : a review

A. Georgala

This study is a review on the fatty acid composition of donkey and camel milk in comparison with other milks. The saturated fatty acid content (SFA) of ruminant species is known to be high (above 70%). Saturated fatty acid content of donkey milk is 59.6% while that of camel milk ranges between 50.15% and 64.12% of the total fatty acids in milk fat. However, donkey milk contains a high amount (14.4%) of polyunsaturated fatty acids (PUFA) in comparison to the 2.3% of cow, buffalo and goat milk or 3.9% of ewe milk. Camel milk is considered as a rich source of long-chain fatty acids (92-99%) and unsaturated fatty acids (35-50%). The predominant fatty acids of camel milk are palmitic and oleic acid. Compared with bovine milk camel milk fat contains smaller amounts of short chain fatty acids. It has been reported that the percentage of saturated fatty acids is higher in bovine milk fat (69.9%) than in camel milk fat (67.7%). Saturated fatty acids (SFA) are reported to have a determinant role in the development of arteriosclerosis, while MUFA and some PUFA are useful to prevent cardiovascular diseases and some inflammatory disorders. Donkey milk has a notable nutritional significance and its fat seems to be more favorable for human health than other milk fats as it contains a lower amount of SFA. Also, it represents an important dietary source of EFA, widely known for the fundamental functions in the human metabolism.

Χημικά χαρακτηριστικά παραδοσιακών Ελληνικών τυριών από τα νησιά του Αιγαίου πελάγους

Chemical characteristics of traditional Greek cheeses of the Aegean Sea islands

A. Georgala

This work studies the chemical characteristics of traditional Greek cheeses of the Aegean Sea islands. Numerous traditional cheeses are made throughout Greece today and twenty of them were recognized as Protected Designation of Origin (P.O.D). Greek traditional cheeses could be grouped according to their technology of manufacture. Cheese samples were taken from different Aegean Sea islands and examined for their pH, moisture, dry matter, fat, fat in dry matter, salt, ash and protein content. Among the cheeses examined were Kopanisti of Chios, Krassotyri of Kimolos/Sifnos, Ladotyri of Milos, Kefalotyri of Ios/Amorgos, Melichloro of Limnos, Xinotyri of Naxos etc. Kopanisti is a soft cheese with a characteristic peppery taste made mainly from cow's in the Cyclades islands. Krassotyri or Possias is a semi–hard cheese made from ovine or caprine milk or mixtures of both, mainly in Kos island, with slightly sour taste and organoleptic properties very much affected of wine sediment (Possia) where the cheese is put after ripening. Kefalotyri is a hard cheese which is characterized by high hardness, salty taste and strong flavour made from sheep's or goat's milk or mixtures of them. Cow's milk may also be used. It is manufactured in various parts of Greece (Kefalotyri of Crete, of Naxos etc). Ladotyri is a hard cheese made from ewe's or mixtures of ewe's and goat's milk raw at home or pasteurized in dairies with a technology very similar to that used for Kefalotyri manufacture, and preserved in olive oil as its name indicates. Xinotyri is a farm hard cheese variety, manufactured from raw goat's milk from indigenous breeds in Naxos island. Melichloro (melipasto) is a hard traditional cheese of Limnos island made from raw ewe's milk at the end of the lactation season. This paper presents the chemical composition of the traditional Greek cheeses of the Aegean Sea showing the differences among the cheese varieties studied.

Aknowledgement : Author would like to thank very much Prof. Anifantakis for his great contribution to this work.

Production of microbial oil from flour-based industrial waste streams

S. Tsakona, N. Kopsahelis, A. Chatzifragkou, S. Papanikolaou, A.A. Koutinas

Flour-based waste streams (FBW) are generated at significant quantities by confectionary industries produced either during processing or as end-of-date products returned from the market. Such waste streams contain high amounts of starch, protein and micro-nutrients and can be utilized as fermentation feedstock. Likewise, utilization of FBW as fermentation media for the production of microbial oil, which can be used as renewable raw material for the production of biodiesel or oleochemicals, constitutes an attractive process.

This study presents the evaluation of hydrolyzed flour-based industrial waste streams as suitable substrate for microbial oil production, using crude amylolytic and proteolytic enzymes produced by a fungal strain of *Aspergillus awamori* through Solid State Fermentation (SSF). The produced hydrolysate was evaluated as fermentation feedstock for the production of microbial oil using an oleaginous strain of *Rhodosporidium toruloides*. Several shake flask fermentations using *Rhodosporidium toruloides* DSM 4444 at different initial glucose concentrations were carried out, aiming at maximization the microbial oil production. Bioreactor fermentations were also carried out using the optimum initial glucose concentration and the obtained results were quite promising. Microbial growth reached up to 28 g/L dry cell weight, with a microbial lipid content of 38% (w/w).

The results of this study indicate that integration of microbial oil production in existing confectionery industries could lead to a viable and sustainable process.

Κατανομή λιπαρών οξέων στο γάλα Ελληνικών φυλών προβάτων

Fatty acids profile of milk from Greek sheep breeds

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Fatty acids profile on sheep's milk was determined in 4 Greek breeds (Karagouniko, Chios, Orini Epirus and Improved Epirus) reared under the same diet and management conditions. Milk samples (n=126) were collected from January to May and fat was extracted from raw milk or butter milk. GC-FID analysis of the lipid revealed the presence of 42 fatty acids (FA). Several lipid quality indices were compared among breeds and levels of lactation stage. Some fatty acids affected significantly by the breed (C16:0, C16-1ω9, C18-3ω-6) and the lactation stage (C14:0, C16-1ω9, C18-2t9t12, C18-4ω-3). Chios breed had higher thrombogenic and hypercholesterolaemic indices than the other breeds. Peroxidisability and atherogenic indices did not differentiate among breeds and lactation stage. A significant increase of ω-3 fatty acids was found in the middle of lactation stage compared with the content of ω-3 at the end of lactation stage.

Principal component analysis applied to 42 variables (fatty acids) revealed that 11 significant factors accounted for 79 % of the variability. Discriminant analysis considering the breed as classification factor, developed two significant discriminating functions with a 80.16% of correct classification. Considering lactation stage as the classification factor, one significant function was developed and the percentage of correct classification was 84.92 %. The fatty acids C18-1cis9, C16:0, C4:0 and C10:0 contributed the most to discriminate milk samples according to breed and lactation stage.

Παραλαβή ελαιοσωμάτων με εφαρμογή υπερδιήθησης και αξιοποίησή τους στην παρασκευή σύνθετων εδώδιμων μεμβρανών καζεϊνικού νατρίου

Oil bodies recovery by applying ultrafiltration and their exploitation for the preparation of composite sodium caseinate-based edible films

A. Matsakidou, M. Tsimidou, V. Kioseoglou

Composite edible films were prepared by incorporating maize germ oil bodies into sodium caseinate solution. Oil bodies in the form of a natural oil-in-water emulsion were extracted from maize germ with water as solvent. The oil bodies dispersion was then subjected in ultrafiltration process in order to concentrate the original dilute emulsion. The obtained "milk" was exploited for the preparation of composite sodium caseinate-based edible films applying the casting technique. Sodium caseinate:oil bodies composite edible films were prepared by drying the film-forming solution that resulted after mixing proper amounts of oil bodies 'milk' and sodium caseinate solution, using glycerol as plasticizer. Droplet size of original oil bodies 'milk' remained unaffected after it was incorporated into sodium caseinate solution. The Attenuated Total Reflectance/Fourier Transform InfraRed (ATR/FT-IR) spectra of the composite films were recorded. A high rate of moisture sorption at short times took place, to finally reach an equilibrium moisture content of all films, during moisture sorption kinetics studies, when sodium caseinate and composite edilble films were kept in stable temperature and relative humidity conditions. Both sodium caseinate and composite films' surfaces were hydrophilic, showing no significant differences between them or between upper and down film sides, revealing that no phase separation took place during film formation. However, rougher surfaces of composite films were shown by micrographs. Sodium caseinate films remained transparent during storage time. On the contrary, composite films were more opaque, while storage time had an opposite effect on their opacity values. Sodium caseinate films performance in tensile test showed a more significant dependence on storage period than oil bodies/sodium caseinate films. In general, composite films presented higher stress-at-break values than sodium caseinate films, as oil bodies incorporation had a plasticizing effect.

Μελέτη παραγόντων που επιδρούν στην ανάπτυξη μυκήτων και στη βιοσύνθεση των καρκινογόνων μεταβολιτών τους

Study of factors affecting fungal growth and the biosynthesis of their carcinogenic metabolites

D. M. Meimaroglou, F. Flouri, D. Galanopoulou, P. Markaki

Aflatoxins, produced as secondary fungal metabolites by some toxigenic strains of the *Aspergillus* species, are known as highly toxic and potent carcinogenic mycotoxins having disastrous effects on human and animal health as well as on agricultural commodities. It is well known that aflatoxin biosynthesis involves lipid peroxidation with the presence of fatty acid hydroperoxides promoting aflatoxin production. Methyl jasmonate (MeJA) is a plant growth regulator as well as the plants response to environmental stress and to wounding by insect and pathogen attack. It derives from α-linolenic acid, which is oxygenized by a LOX-enzyme to 13S-hydroperoxylinolenic acid. Fungal attack by certain *Aspergillus* species is likely to induce the synthesis of this secondary metabolite. Several experiments using Petri dishes have been conducted in our laboratory in order to elucidate the inhibitory effect of this bioregulator on AFB_1 production by toxigenic fungi. The present study reports of the effect of MeJA on the AFB_1 output in an edible plant of Greek origin used as a substrate, namely caper. According to our experiments in a nutrient medium of well defined composition (YES) where MeJA was added at 10 different concentrations (10^{-1}M-10^{-6}M MeJA/10mL YES), the addition of the lowest amount of MeJA (10^{-6}M) resulted in the stimulation of fungal growth and AFB_1 production concomitantly, whereas MeJA added at the higher concentrations (0,004M, 0,008M, 10^{-2}M, 0,016M, 10^{-1}M/flask) inhibited both *A.parasiticus* growth and AFB_1 production during the entire incubation time of 20 days. Based on our experiments in YES, 5 different MeJA concentrations were chosen for the caper substrate (10^{-1}M, 10^{-2}M, 10^{-3}M, 10^{-4}M and 10^{-6}M MeJA/15g caper). Similarly to the YES results, MeJA added at the lowest concentration (10^{-6}M) significantly stimulated AFB_1 production after the 9^{th} day of incubation. The addition of MeJA at the other concentrations either significantly decreased (10^{-4}M, 10^{-3}M) or inhibited AFB_1 production during the entire incubation time (15 days). The highest concentrations of MeJA 10^{-2}M and 10^{-1}M inhibited AFB_1 by 97,74% and 98,42% on the 12^{th} day of observation respectively.

Μελέτη της αντιοξειδωτικής δράσης τριών φαινολικών ουσιών σε φυτικά έλαια

Antioxidant activity of three natural phenoliic antioxidants in various vegetable oils: a comparative study

E. Vagena, I. Papadaki, D. Tsimogiannis, V. Oreopoulou

Natural phenolic antioxidants have gained increased interest because of their scavenging activity towards free radicals and other reactive species that promote lipid oxidation in foods. Lipid substrates, such as vegetable oils, are prone to oxidation processes depending on their unsaturated fatty acid profile, tocopherol and metal content. Catechin, quercetin and rosmarinic acid are phenolic compounds naturally occurring in plants that show a pronounced scavenging activity towards free radicals and are expected to prevent lipid deterioration and increase the shelf life of lipid containing products. In this study, the stabilization of different vegetable oils with these compounds has been evaluated. Corn oil, cotton oil, sunflower oil and soybean oil samples have been subjected to accelerated oxidation at 60 ^{o}C for a period of 3 weeks. Comparative results are demonstrated and correlated to fatty acid profile, tocopherol and metal content of these oils. Moreover, a correlation of the activity of the three antioxidants with their structure and solubility in the oils is attempted.

Ανίχνευση και ταυτοποίηση φαινολικών συστατικών σε δείγματα έξτρα παρθένου ελαιόλαδου με HPLC-DAD και GC-MS

HPLC-DAD and GC-MS analysis of phenolic compounds in extra virgin olive oils

Ch. Proestos, M. Komaitis, P. Zoumpoulakis, V. Sinanoglou

Phenolic compounds in Greek olive oils were analyzed by reverse phase high performance liquid chromatography (RP-HPLC) with diode array detector (DAD) and by GC–MS after extraction with methanol:water 80:20 and derivatization with bis (trimethylsilyl) trifluoracetamide and trimethylchlorosilane (BSTFA:TMCS 99:1). Tyrosol, hydroxytyrosol and the decarbomethoxy ligstroside and oleuropein aglycons in the dialdehydic forms were the most abundant compounds. 3,4 dihydroxyphenylacetaldehyde, syringaldehyde, the cis form of ferulic acid, *p*-hydroxybenzoic acid, caffeic acid and luteolin were identified. Phenolic compounds, mainly polyphenols are bioactive compounds in virgin olive oil and they have been related to the stability of the oil. Several reports mention natural antioxidants such as polyphenols in olive oil for their antivascular and antimicrobial effect on human health. Total phenolics were also determined by the Folin-Ciocalteu colorimetric method. Fatty acids in the examined samples were determined by gas chromatography (GC) with FID detection.

Εκτίμηση, αξιολόγηση της οξείδωσης και σταθερότητας λιπαρών υλών-ελαίων με τη μέθοδο Rancimat. Μελέτη της αντιοξειδωτικής δραστικότητας φυτικών εκχυλισμάτων και αιθερίων ελαίων

Antioxidant capacity of plant extracts and essential oils by the Rancimat test. Determination of lipid oxidation and stability

Ch. Proestos, M. Komaitis, P. Zoumpoulakis, V. Sinanoglou

In an effort to minimize the undesirable effects of the synthetic food preservatives in human health, food industries and scientists have recently turned the interest to new preservatives. Aromatic plants are well known for their antioxidant and antimicrobial properties that prevent food degradation and alteration, as they are rich in phenolic substances, usually referred as polyphenols, which are ubiquitous components of plants and herbs. The antioxidant capacity was determined, in dried plants and in their methanol extracts and essential oils, with the Rancimat test using sunflower oil as substrate. The method used is the official method of AOCS, Cd 12B-92 for lipid oxidation determination. All grounded plants, essential oils and their extracts showed antioxidant capacity. The antioxidant capacity was expressed as PF values. The outcome of the Rancimat test supports the claim that aromatic plants are good sources of natural antioxidants such as phenolic compounds. When ground material was added to sunflower oil, protection factors were slightly higher compared to the addition of methanol extracts and essential oils.

Παραγωγή βιοαιθανόλης κατά την αύξηση της ζύμης *Saccharomyces cerevisiae* MAK 1 σε μίγματα μελάσας και υγρών αποβλήτων ελαιουργίας υπό μη-στείρες συνθήκες

Bio-ethanol production during growth of the yeast *Saccharomyces cerevisiae* MAK 1 on mixtures of molasses and olive mill wastewaters under non-sterile conditions

D. Sarris, L. Matsakas, A. A. Koutinas, M. Komaitis, S. Papanikolaou

Olive mill wastewaters (OMWs) are the liquid residues of the olive oil industry, considered to be as one of the most difficult to treat agro-industrial wastes due to their high content in phenolic compounds. Molasses are the by-product of sugar industry consisted of high total sugars and mineral content, having strong odor and dark color because of the existence of the high molecular weight polymer pigment melanoidin. *Saccharomyces cerevisiae* MAK 1 was cultivated on mixtures of molasses and OMWs in shake-flask and bioreactor cultures under completely non-sterile conditions. Remarkable decolorization (up to 60%) and non-negligible removal of phenolic compounds (up to 28% *w/w*) occurred in all trials. In several cases, high initial phenolic compounds concentrations were observed (up to ~6.5 g/L) without important inhibition exerted. In shake-flasks, adaptation to molasses media supplemented with OMWs, not significantly decreased biomass and ethanol production compared with the control experiments (no OMW added – culture performed only on molasses). Ethanol was produced up to 34.3 g/L (yield of ethanol produced per total sugar consumed, $Y_{EtOH/S}$~0.40 g/g), while biomass up to 7.3 g/L (yield of biomass produced per total sugar consumed, $Y_{X/S}$~0.08 g/g). In non-sterile bioreactor cultures, biomass production (up to 5.3 g/L, $Y_{X/S}$~0.06 g/g) was reduced and ethanol production was significantly enhanced (up to 52.4 g/L, $Y_{EtOH/S}$~0.48 g/g), comparing with flask experiments. No supplementation of pure sugars and salts, use of low volumes of water for dilution, no aeration during fermentations and no sterilization can lead to tremendous reduction of the bioprocess cost.

Μελέτη λιπιδικού προφίλ του εδώδιμου μύκητα *Laetiporus Sulphureus*

Lipid profile study of the edible fungus *Laetiporus Sulphureus*

V.J. Sinanoglou, P. Zoumpoulakis, J. Petrovic, J. Glamoclija, M. Sokovic, Ch. Proestos, G. Heropoulos

Laetiporus sulphureus is a species of bracket fungus found mainly in Europe and North America. Its common names are sulphur polypore, sulphur shelf, and chicken of the woods because of its taste which may also resemble the taste of crab or lobster. Its fruit bodies grow as golden-yellow shelf-like structures on tree trunks and branches. Like other bracket fungi, they may last many years and fade to pale grey or brown. The under surface of the fruit body is made up of tubelike pores. Laetiporus sulphureus is a saprophyte and causes brown cubical rot in the heartwood of trees on which it grows. Unlike many bracket fungi, it is edible when young.

In this study, the lipid profile of the fruiting bodies of L. sulphureus has been studied using GC-FID and Iatroscan TLC FID methodologies. Different types of extractions including high energy techniques were employed to identify the optimum conditions for higher yield of lipid isolation. Optimum extraction methodologies provided two fractions, one containing neutral and polar lipids (TGs, sterols, GL, phospholipids) and the other mainly attributed to fungal carotenoids and pigments. The existence of relatively high amount of sterols may be correlated to fungus pharmaceutical properties. Fatty acid analysis indicated a predominant level of polyunsaturated fatty acids (PUFA) followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA).

Μεταβολές στην ποιότητα ημι-διατηρημένων ωοθηκών *Mugil cephalus* (αυγοτάραχο), κατά τη διάρκεια της αποθήκευσης σε 3,0 ± 1,0 ºC

Quality changes of semi-preserved *Mugil cephalus* ovaries (avgotaracho), during storage at 3.0 ± 1.0 ºC

V.J. Sinanoglou, M. Voulgarelis, P. Androutsaki, I.F. Strati, V. Oreopoulou, V.P. Lougovois

Quality changes of Greek avgotaracho were assessed over a 4-month storage period at 3.0 ± 1.0 ºC, by lipid, fatty acid and carotenoid profile analysis, assays of thiobarbituric acid-reactive substances (TBA-RS), colour measurements (*CIE L*a*b**) and determinations of total volatile bases (TVB). The major lipid classes in freshly prepared avgotaracho were neutral lipids (NL), mainly triglycerides (TG), followed by cholesterol. Polar lipids (PL) consisted mainly of phosphatidylcholine (PC), followed by sphingomyelin (Sphm) and phosphatidylethanolamine (PE). Cold storage did not significantly affect the individual lipid classes' content. The minor amounts of free fatty acids and monoglycerides released over the storage period suggested slow TG and PhL hydrolysis. GC-FID analysis of the lipid revealed the presence of 37 fatty acids (FA). Heptadecanoic acid (C17:0) was the main saturated fatty acid (SFA), followed by palmitic acid (C16:0), while palmitoleic (C16:1ω-9), oleic (C18:1ω-9) and vaccenic (C18:1ω-7) were the major monounsaturated fatty acids (MUFA). Polyunsaturated fatty acids (PUFA) occurred in the lowest concentration and consisted mainly of DHA (C22:6ω-3) and EPA (C20:5ω-3). An increase in SFA content and a decrease in PUFA, especially ω-3 fatty acids, was observed during storage. PUFA/SFA, MUFA/SFA and ω-3/ω-6 ratios gradually decreased in the stored product. However, oxidative changes were rather limited, as judged by the slow increase in TBA-RS levels. HPLC-DAD analysis of the carotenoids showed that *β*-cryptoxanthin predominated, followed by *all-trans* lutein, *all-trans* canthaxanthin, *α*-cryptoxanthin and *all-trans* zeaxanthin. Carotenoid profile was not affected by storage. Nevertheless, a significant decrease in individual carotenoid compounds was observed in the stored product. *CIE L*a*b** coordinates varied considerably between samples, probably reflecting differences in roe maturation. The high levels of volatile basic nitrogen (up to 133.98 mg/100 g) observed after 4 months of storage are in accordance with previous results in this field of study and appear to be associated with autolytic and microbial activities during the ripening process.

Μελέτη εκχύλισης ελαιοπυρήνα και βιοδραστικών του συστατικών με χρήση μιγμάτων διαλυτών διαφορετικής πολικότητας

A study of olive kernel oil extraction and bioactive compounds recovery using mixed-polarity solvents

S. Chanioti, C. Tzia

Olive oil production represents an economic and social industrial activity that is highly relevant in the South European countries. One of the main by-products remaining after the olive oil manufacturing process (either by pressing or by centrifugation) is olive kernel. Olive kernel is a residue consisting of pieces of pulp, pit, skin of the olive fruit and varied moisture content. Great quantities of olive kernel are delivered containing oil that can exceed 10%. Therefore, olive kernel utilization is of significant economic interest, while in addition olive kernel oil presents very similar composition to that of olive oil. The Soxhlet extraction technique has become the most used tool for solid-liquid extraction in many fields. It constitutes the common method for oil extraction from olive seeds in oil industry. The use of the extraction by solvent allows olive kernel's valorization by recovery of its residual oil. The performance of an extraction process is governed by both mass transfer and equilibrium phenomena. The oil extraction rate is influenced by a number of factors, including the thickness or the size of the seed particles and the intrinsic diffusion capacity of solvent and oil. Thus, the solvent selection is considered the determinant factor for the extractability that can be investigated for the process optimization for both efficiency and economic reasons. The aim of this study was to examine the extraction of olive kernel oil and of its individual bioactive compounds such as sterols and squalene using mixed-polarity solvents (petroleum ether, hexane, diethyl ether, isopropyl alcohol, ethyl acetate). The particle size of the olive kernel on oil and total unsaponifiable matter recovery was studied as well.

Μελέτη της έκκρισης της λιπάσης των μονοακυλογλυκερολών και της αμιδοϋδρολάσης των λιπαρών οξέων στην *Tetrahymena thermophila*

Secretion of monoacylglycerol lipase and fatty acid amide hydrolase in *Tetrahymena thermophila*

A. Stamogiannos, E. Gkini, D. Galanopoulou, A. Velentzas, M. Antonelou, I. Papassideri, A. Siafaka-Kapadai

In the present study, the secretion of the two main degradative enzymes of the endocannabinoid system, the fatty acid amide hydrolase (FAAH) and the monoacylglycerol lipase (MAGL), in the protozoan *Tetrahymena thermophila* was investigated. *Tetrahymena* has been regularly used as a model organism for the study of secretion and has two well characterized routes of secretion: the constitutive secretion of lysosomal enzymes and the regulated secretion of mucocysts. Initially, the secretion of both enzymes was investigated in starvation medium, using [^{3}H]2-oleoylglycerol (2-OG) as substrate and in the presence or absence of FAAH specific inhibitor AM374. Both enzymes were secreted in the starvation medium, in a time dependent manner. The maximum secretion was ~5 % after 4 hours incubation and steadily declined afterwards. The secretion of these enzymes is a combined result of two different sources of constitutive secretion: a lysosomal and a non-lysosomal pathway. In the case of FAAH, the non-lysosomal pathway seems to be specific. Subsequently, stimulation of secretion with dibucaine leads to exocytosis of the mucocysts of *Tetrahymena.* In the isolated mucus MAGL and FAAH activity were determined, using [^{3}H]2-OG as substrate in the presence of AM374 or MAGL specific inhibitor JZL184. In the case of MAGL, the activity measured was equivalent to that of the cell homogenate. Supernatant was enriched in FAAH activity, confirming our results about the existence of a non-lysosomal secretion pathway for this enzyme. Immunoblot analysis using anti-MAGL antibody revealed the presence of an immunoreactive protein at ~45kDa in the mucus fraction, the supernatant and homogenate. Finally, confocal laser scanning microscopy (CLSM) showed mostly pericellular localization of MAGL, suggesting its existence in the mucocysts.

Acknowledgements: This work was supported in part by the University of Athens Special Account for Research Grants (70/4/3351).

Επίδραση του συστήματος και των συνθηκών εκχύλισης, χρόνου και θερμοκρασίας μάλαξης, στα ποιοτικά χαρακτηριστικά του ελαιολάδου

Effect of extraction system and conditions, malaxation time and temperature, on the quality characteristics of virgin olive oil

K. Kotsiou, K. Gennatos, M. Tasioula-Margari

The aim of this work was to study the phenolic and volatile compounds of oils extracted from homogeneous batches of olive fruits (Lianolia, Asprolia and Koroneiki varieties) by different processing conditions. The examined parameters were malaxation time (30-80min) and temperature (24 °C and 32 °C) in two and three phases centrifugation systems.

Temperatures of 24 °C and 32 °C did not show any significant effect in individual and total volatile compounds processed in two or three phases centrifugation system. On the other hand, a relatively low increase (up to 22%) in total phenolic content was observed with hydroxytyrosol derivatives being mainly affected.

Contrasting results have been obtained on the effect of malaxation time on the volatile composition. C6 aldehydes did not show any specific trend while alcohols, esters and ketones did not seem to be affected. However, phenolic content increased up to 24% in oils processed at high temperature.

It could be concluded that under the studied range of malaxation time and temperature, changes that might occur in volatile compounds cannot be considered significant. The observed increase in phenolic content with increasing temperature and time is concentration dependant. Hydroxytyrosol derivatives were mainly affected, followed by tyrosol derivatives, while lignans and simple phenols were not affected.

Acknowledgements: *The study is financially supported by the European Territorial Cooperation Programme "Greece-Italy 2007-2013".*

Antiproliferative action of pumpkin seed lipid extracts on PC-3 prostate cancer cells

R. Tenta, M. Xanthopoulou, M. Tsoukala, H. Pratsinis, D. Kletsas, S. Antonopoulou, T. Nomikos

Pumpkin seed oil is traditionally used for the treatment of benign prostate hyperplasia and human trials confirmed this observation. However, the exact mechanisms underlying this protective effect are not well understood. PC-3 is an androgen insensitive human prostate cancer cell line, widely used for the study of prostate cancer. Aim of the present study was to evaluate whether pumpkin seed extracts affect the proliferation and the cell cycle distribution of PC-3 cells.

Different extraction procedures were applied to commercially available pumpkin seeds. PC-3 cells were treated with the extracts and the inhibition of cell proliferation was evaluated by the MTT assay. Cell cycle distribution was assessed by flow cytometry. Adriamycin, producing G2/M arrest and apoptosis of PC-3 cells, served as a positive control. Autophagy was assessed by immuofluorescence.

The screening of six different extracts showed inhibition of PC-3 cells proliferation by 96h exposure in a dose-dependent manner (1-1000μg/mL). The two most potent extracts [polar lipid extract (PL) and water extract (W)] were further tested. Treatment of the PC-3 cells with 150μg/mL of PL for 48 hours increased the cell distribution in S phase (24%), while treatment with 200μg/mL of W did not alter cell distribution. Finally, treatment with 100 nM of adriamycin showed the expected G2/M phase blockade (85%). Although the extracts tested did not produce evidence of apoptosis, they produced evidence autophagy induction in a dose-dependent manner.

Herein, we show that pumpkin seed extracts have direct effects on the proliferation and survival of PC-3 cells, which may have important implications for the design of a more effective adjuvant treatment for prostate cancer patients.

Antiproliferative effects of red and white wine extracts in PC-3 prostate cancer cells

R. Tenta, M. Xanthopoulou, M. Tsoukala, H. Pratsinis, D. Kletsas, S. Antonopoulou, E. Fragopoulou

Background: Bioactive food components are increasingly being evaluated as potential prostate cancer chemopreventive agents. The goal of the present study was to evaluate whether wine extracts, already established for their ability to inhibit lipoxygenase activity, lipid peroxidation and their antiradical activity, affect the proliferation and the cell cycle distribution of PC-3 prostate cancer cells.

Materials and Methods: Cabernet Sauvignon-(CS) and Rombola-(R) wines were extracted with two different methods in order to obtain either different lipid fractions (total, polar, neutral) or several fractions containing different classes of phenolic compounds (FI: Anthocyanins, FII: Procyanidins, catechins, flavonols, FIII: Phenolic acids, quercetin 3-O-glucuronide, FIV: The rest phenolic components). PC-3 cells were treated with the extracts or standard compounds (resveratrol, quercetin, gallic acid, tyrosol) (1-1000μg/mL) and the inhibition of cell proliferation were evaluated by the MTT assay. Cell cycle distribution was assessed by flow cytometry. Adriamycin, producing G2/M arrest and apoptosis of PC-3 cells, served as a positive control. Autophagy was assessed by immuofluorescence.

Results: All extracts inhibited PC-3 proliferation in a dose-dependent manner. The most potent compound of each group [CS-FII, R-FII, Resveratrol] was selected to be further tested. The cell cycle distribution revealed that treatment of the PC-3 cells with 150μg/mL of CS-FII for 24h and 48h or R-FII for 72h marginally increased the cell distribution in S phase, while resveratrol (15μg/mL) increased the distribution of cells in G0/G1 phase (82%). Although the extracts tested did not produce evidence of apoptosis, the fraction of procyanidins, catechins, and flavonols produced evidence of autophagy induction.

Conclusions: **CS and R extracts strongly inhibit PC-3 cell proliferation. Our data are very promising, regarding the application of these extracts in the field of prostate cancer.**

Αντιαιμοπεταλιακή δράση και περιεκτικότητα φυτοστερολών σε λιποειδικά εκχυλίσματα ξηρών καρπών

Antiplatelet effect and phytosterol content of nuts' lipid extracts

I. C. Vlachogianni, N. Kalogeropoulos, S. Antonopoulou, C. A. Demopoulos, T. Nomikos

Increased platelet reactivity and platelet-activating factor (PAF) are crucial predisposing factors for atherosclerosis development and dietary extracts possessing antiplatelet and anti-PAF activity may confer protection from cardiovascular diseases (CVDs). Epidemiological studies imply that a moderate consumption of nuts has beneficial effects on CVD rates mainly due to the lipid content of them. However, the antiplatelet activity of nuts' lipid extracts has not been studied extensively before. Under this perspective, the inhibitory properties against PAF-induced platelet aggregation of lipid extracts derived from nuts (walnut, almond, pistachio, pumpkin and sunflower seeds) traditionally consumed among Greeks were tested.

The total lipids (TL) were extracted using the Folch method. Polar (PL) and neutral lipids (NL) were separated with countercurrent distribution. Total lipidic phosphorus was determined in TL and PL while identification and quantification of sterols was conducted in TL. All three lipid classes were studied for their ability to aggregate washed rabbit platelets or inhibit PAF-induced platelet aggregation.

The extracts from all nuts inhibited platelet aggregation. PL from almond showed the best inhibitory activity with an IC_{50} of 0.049 μg/μL platelets, followed by TL and NL of sunflower seeds. The fat of all nuts contained significant amounts of phytosterols (2-5 mg/g fat), pistachio fat had the higher concentration of phytostrerols. The majority of phytosterols was found in the form of b-sitosterol. The inhibitory activity of nut extracts was positively correlated with the content of stigmasterol.

The relatively strong antiplatelet and anti-PAF activity of nuts' lipid extracts along with their high content of phytosterols may partly explain their cardioprotective properties.

Παραγωγή λιπιδίων κατά την αύξηση της ζύμης *Cryptococcus curvatus* σε εμπλουτισμένα με λακτόζη υγρά απόβλητα ελαιουργίας

Lipid production during growth of the yeast *Cryptococcus curvatus* on lactose-enriched olive mill waste-waters

E. Xenopoulos, A. Chatzifragkou, A. A. Koutinas, S. Papanikolaou

Olive oil mill waste water (OMW) is a difficult to treat effluent due to its dark color and its phytotoxic properties. Cheese-whey is by-product from cheese production industries that presents high BOD/COD values. OMWs and lactose deriving from cheese-whey were used as a substrate for the growth of *Cryptococcus curvatus* ATCC 20509 in order to assess its potential to produce lipid amenable to be converted into biodiesel in shake-flasks. Lactose and salts solutions and OMWs were blended in order to create nitrogen-limited media presenting similar initial lactose concentrations (~40 g/L) and different initial phenolic compound concentrations (0.0 – blank, 1.0, 1.5, 2.0 and 2.5 g/L). As the phenolic compounds concentration increased, biomass and lipid production where lower compared with the control experiment; in the blank experiment, maximum total biomass and lipid quantities were 12.0 and 3.8 g/L respectively with almost all of the available lactose being consumed (within ~200 h), whereas in the trial with 2.5 g/L of phenolics, biomass and lipid quantities of 5.5 and 1.5 g/L with notable lactose quantities remaining unconsumed (within ~200 h) were obtained. However some detoxification of the residues was reported as removal of phenolic compounds and color (~25%) was reported. In media with lower initial phenolic compounds concentrations (e.g. 1.0 g/L) biomass and lipid production were slightly affected (maximum values 11.0 and 2.2 g/L respectively) whereas even higher removal of phenolic compounds and color (36% and 27% respectively) were observed. Lipids containing principally oleic and palmitic acid, suitable for biodiesel generation, were synthesized.

Σύνθεση των λιπαρών οξέων και των στερολών Ελληνικών παραδοσιακών προϊόντων με βάση το γάλα και τα δημητριακά

Fatty acid composition and sterol content of Greek traditional Milk-cereal foods

C. Chatzi, E. Zoidou, T. Massouras

Traditional milk/cereal foods (e.g. trachanas, noodles) are dried foods, based on a mixture of ground wheat or semolina and raw or acidified milk in the ratio (2:1) respectively. Occasionally instead of milk a pulp of vegetables is used. So depending on the raw materials, various types of products will be obtained. These products are highly nutritious and minor constituents that are deficient in milk are supplemented by cereal and vice versa. The present study aimed at investigating the lipid fraction (fatty acids profile and cholesterol) of traditional milk/cereal foods. Different kind of these products: sweet and sour Trachanas, noudles and trachanas with vegetables pulp called “nistisimos” were analyzed. The chemical composition and the nutritional value of Trachanas mainly depend on the milk and the wheat added during the production process, as well as the proportion of these two ingredients in the recipe. Dietary fiber content ranged from 0.58% for the noodle with milk to 2.22% for trachana made from whole wheat flour, while trachanas “nistisimos” had intermediary dietary fiber content (1.17%). The most important fatty acid from a quantitative viewpoint was palmitic acid (16:0), which ranged from 23.89 to 29.87% by weight of the total fatty acids. Myristic acid (14:0) and stearic acid (18:0) make up 8.3-13.2 and 14.48.48 to 27.67% by weight, respectively. The contents of saturated fatty acids SFA(%), monounsaturated MUFA (%) and polyunsaturated fatty acids PUFA(%) as percentage of the total fatty acids were found to be 63.90 ± 0.62, 26.96 ± 0.94 and 11.46 ± 0.01, in all the type of ~~t~~rachana made by milk~~,~~ 46.2 ± 0.36, 48.20 ± 0.76 and 5.81 ± 0.06 in trachanas “nistisimo”, 47.76 ± 0.30, 36.69 ± 0.84 and 15.69 ± 0.01 in noodle with milk, respectively. The ratio of plant sterols to cholesterol was found to be 1.32 and 46.6 for trachanas with and without milk “nistisimo” respectively.

Μεταβολή στην σύσταση των λιπαρών οξέων του πρόβειου γάλακτος μετά από χορήγηση διατροφικών συμπληρωμάτων εσπεριδίνης

Variation in fatty acid composition of ewe‘s milk during dietary supplementation with hesperidin

K. Moschou, T. Massouras, E. Zoidou, S. Deligiorgis, J. Bizelis

The aim of the present work was to evaluate the possible effects of hesperidin dietary supplementation on ewe’s milk chemical characteristics and especially on fatty acid composition. 15 lactating Karagouniko breed ewes were divided into three groups; one group was fed with a commercial concentrated diet supplemented with 1g hesperidin / kg, the second was fed with concentrated diet supplemented with 3 g hesperidin / kg and the third group was the control. During the 15 days pre-experimental period ewes were adapted to their new diet and the experimental period lasted 45 days. Individual milk samples drawn on 0d, 15d, and 45d were analyzed for chemical composition (protein, lactose, fat and total solids) and those drawn on d 45 were also subjected to HPLC and SPME/GC/MS analysis for the determination of hesperidin and fatty acids profile respectively. The results showed that the incorporation of hesperidin in the diet of lactating ewes, caused a significant increase ($P < 0.05$) in the level of butyric acid (C4) in milk, the greater amount determined in 45d samples. In contrast, palmitoleic acid (C16:1) proportions had a significant decrease during the time. Chemical characteristics of ewe’s milk did not differ significantly among the three groups. Hesperidin was detected in low amounts in ewe’s milk from both ~~the~~ experimental groups. However, similar experiments with this substance have not been done yet and as a result hesperidin offer a fertile sector for further research.

Μελέτη αντιοξειδωτικών και *in vitro* αντιοξειδωτικής δράσης ελαιολάδου,κατά την ωρίμανση ελιών ποικιλίας «Κορωνέϊκη»

Study of olive oil antioxidants & in vitro antioxidant activity, during ripening process of olives var. "Koroneiki"

A. Artemiou, N. Kalogeropoulos, A. Kaliora, A. Chiou

Olive oil, the main source of fat in the Mediterranean diet, is considered responsible for the low incidence of atherosclerosis, cardiovascular diseases and cancer. In the present study quality indices, antioxidant activity and functional properties of olive oils were followed during the maturation of olives of the *Koroneiki* cultivar, grown in an olive grove at the Messinian Mani, Peloponnese. More specifically, olive oils were examined for acidity, UV absorbance (K_{270}, K_{232}), peroxide value, total phenolic content, fatty acids profile, $DPPH^{\bullet}$ radical scavenging capacity and ferric ion reducing antioxidant power (FRAP). In addtition, *in vitro* tests for the inhibition of inflammatory markers (TNF-α, MCP1, IL-6) in stimulated human mononuclear cells (PBMCs) and serum lipid oxidation by olive oils methanolic extracts were also assayed.

The ripening of olive fruits resulted in the following changes of the obtained oils:

- total phenolics, squalene, and ferric reducing power increased
- terpenic acids and tocopherols decreased
- monounsaturated fatty acids increased and polyunsaturated fatty acids decreased
- the polar extract of oils from mature olive fruits inhibited serum lipid oxidation and inflammatory markers in PBMCs more effectively than oils obtained from less ripened olives

As a conclusion, for the specific cultivar and geographical area, it is recommended to pick up olives when they are mature, as they are expected to provide a higher quality olive oil, with additional benefits for human health. Nevertheless, further research should be carried out on the effects of pedoclimatic conditions, oil production and storing conditions on the quality of *Koroneiki* olive oil from Messinian Mani.

Επίδραση του μεγέθους στα οργανοληπτικά χαρακτηριστικά και τη σύσταση καλλιεργημένων κρανιών (*Argyrosomus regius*)

Effect of size on the sensory characteristics and fillet composition of farmed meagre fish (Argyrosomus regius)

J. Giogios, K. Grigorakis, N. Kalogeropoulos

The common meagre (*Argyrosomus regius*) is an important candidate for diversification of the Mediterranean mariculture; it is a fast-grower, exhibiting no major reproduction and feeding problems and is reared for more than a decade.

Little knowledge exists on the quality of this fish species, while in addition there is a market rumor that meagre smaller than 1Kg has profoundly inferior quality. Hence, this study aimed to compare the proximate composition, fatty acids and volatile compounds in the flesh of 2 meagre groups, differing in size, with average weights equal to 0.7 and 1.3 kg, respectively.

Size did not affect the proximate composition of the two groups, their fat and protein contents being 0.7-1% and 20%, respectively. Differencews were observed in fillet fatty acids, with the larger fish containing significantly higher ω6 polyunsaturates (16.4% vs. 15.0%, respectively, p=0.002) and slightly lower eicosapentaenoic (EPA) (p=0.10), indicating nutritional quality differentiations with size.

Volatile aroma compounds from fillets of both cultured and wild meagre fish, were isolated by simultaneous steam distillation-extraction and analysed by GC-MS. Analysis resulted in the identification and quantification of 71 volatile compounds, which exerted differences and similarities which are discussed.

In addition a sensory panel trial confirmed that the bigger specimens exhibit better organoleptic characteristics.

Lipid microemulsions and their potential as delivery systems for bioactive compounds

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There has been considerable interest in using lipid microemulsions to encapsulate bioactive components. It is worth noting that lipids can be produced by natural sources such as plants, animals and microemulsions can find innovative utilization in the food and beverage industries for certain applications. Moreover, microemulsions are increasingly being utilized in consumer products, mainly in pharmacy and cosmetics. This happens because of their unique physicochemical and functional properties: high encapsulation efficiency, low viscosity and turbidity and high stability. The purpose of the present study was to formulate microemulsions as final application products. More specifically, the objective was the preparation of thermodynamically stable microemulsion systems having a large interfacial area and solubilization capacity.

Having a formulated microemulsion, various substances with biological activity can be incorporated either by adding them gradually with the aqueous phase or by mixing them initially with the oil phase. Of particular interest is the incorporation of lipophilic compounds such as drugs, antioxidants, flavors. The use of microemulsions is foreseen as an effective carrier to overcome the solubility limitations. For this, the system should be biocompatible and owe a diluting capacity against water.

The components that were used are safe and biocompatible materials. Initially, different systems were developed consisting of natural oils (R-(+)-Limonene), non-ionic surfactants or mixtures of them (Tween 20, Tween 40, Tween 40:Tween 20 (1:1), Tween 40:Tween 20 (2:1)), distilled water and propylene glycol as the aqueous phase. The phase behavior of these systems was described by pseudo-ternary phase diagrams, which were determined at 25 oC. Depending on the microemulsion system (o/w or w/o or bicontinuous), various bioactive compounds owing different hydrophilicity were incorporated. Compounds as Squalene (lipophilic compound), Octyl gallate (amphiphilic compound) and Gallic acid (hydrophilic compound) were successfully tested. Furthermore, structural characterization of the proposed empty and loaded microemulsions is of great importance regarding their future applications. In this respect various techniques, such as Electron Paramagnetic Resonance (EPR) spectroscopy, Dynamic Light Scattering (DLS) and electrical conductivity were carried out to characterize the microemulsion formulations.

Διεπιφανειακές ιδιότητες λιπαρών υλών και η σχέση τους με την τεχνολογία και τον έλεγχο ποιότητας

Interfacial properties and their relation with the technology and quality in dietary oils and fats

E.P. Kalogianni

This work presents rare data on dietary oil interfacial properties. Interfacial properties are examined as a function of different processes such as refining of olive pomace oil and frying using different oil types and conditions. Furthermore, the interfacial properties are determined for oils having different qualities. Interfacial properties are not customarily used as means for quality control in a process chain involving oil or as methods for the determination of oil quality. However, our results show that dynamic interfacial tension measurements can provide valuable information. A discussion is made on the potential use of these measurements for quality control. Finally, the possibility of differentiation between different oil qualities (including olive oil) is considered.

Κριτήρια απόρριψης επαναχρησιμοποιούμενων τηγανέλαιων με χρήση της τριχοειδούς αναρρίχησης σε πορώδη μέσα

Capillary rise in porous media to set rejection criteria for reused fried oils

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The intense and complex heat and mass transfer processes during deep fat frying result in significant oil degradation which imposes oil replenishment in sequential frying batches. The determination of the exact instant that frying oil must be replenished is a major concern for avoiding possible health risks but also for estimating the cost of fried foods in food industry and catering applications. This work investigates the potential of setting the fried oil rejection criteria by employing the phenomenon of capillary rise of oil into a porous medium. To achieve this goal, wicking patterns (oil penetration rate and oil front shape versus time) of both fresh and prolonged fried oils are optically registered at six different paper sheets used as porous media. Four of them are double-ply towel papers whereas the other two are single-ply chromatographic papers. Wicking tests are performed at 20^{o}C and 30^{o}C. The data of the present study show that the type of paper affects seriously the wicking patterns. Double-ply papers present high oil penetration rates but very irregular oil front shapes whereas single-ply papers yield lower oil penetration rates but pretty flat oil fronts. Furthermore, it is found that only under certain conditions the penetration rates obey the well known Lucas – Washburn equation. A discussion is made on the phenomena that take place during wicking of oil into paper which may cause deviations from the Lucas – Washburn equation. A semi-empirical model is proposed to describe the above deviations by incorporating the effect of time evolving pore sizes.

Χρήση ρευστοποιημένων ξηρών στελεχών γλυκού σόργου για την παραγωγή λιπιδίων από κύτταρα *Lipomyces starkeyi* CBS 1807

Use of liquefied dry sweet sorghum stalks for the production of lipids by Lipomyces starkeyi CBS 1807 cells

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Sweet sorghum (Sorghum *bicolor* {L.} Moench) is a high biomass- and sugar-yielding crop. It contains approximately equal quantities of soluble (glucose, fructose and sucrose) and insoluble carbohydrates (cellulose and hemicellulose) and has been considered as an important source for the production of second generation biofuels.

Aim of this work was to examine the ability of the utilization of liquefied dry sweet sorghum stalks as a raw material for cultivating *L. starkeyi*, in order to accumulate lipids. During the first experiments, *L. starkeyi* cultivated on synthetic media consisted by commercial sugar solutions, in order to examine the effect of different factors (such as different sugars, nitrogen sources and different C:N ratios) on lipid accumulation. Under optimized conditions and initial sugars concentration of 40 g/L lipid production reached 5.8 g/L, which was equivalent to a lipid accumulation of 47,7% of yeast dry weight.

Finally, *L. starkeyi* cultivated on dried sweet sorghum stalks (which served as both carbon and nitrogen source) at an initial dry material concentration of 10%. During this process an enzymatic prehydrolysis step was included, using commercial cellulases (Celluclast 1.5L) and β-glucosidase (Novozym 188), which rapidly reduces the viscosity (or flowability) of the high solid content substrate and enables better mixing for the inoculation of the fermenting organism. Due to the fact that Novozym 188 exhibits significant invertase activity (0.83 Unit/mg protein), only Celluclast 1.5L was added at the liquefaction step, in order not to hydrolyze sucrose and therefore increase glucose inhibition to commercial cellulases. Novozym 188 was added at the start-up of fermentation in order to further hydrolyze cellobiose to glucose.

Monoolein production under High-Pressure Vapor-Liquid Equilibrium

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Surfactants when used in food, pharmaceutical and industrial applications should be biodegradable, biocompatible and non-toxic. They are commonly produced through alkaline-catalyzed chemical glycerolysis of natural oil and fats at high temperatures and elevated pressure under nitrogen atmosphere. The purpose of this study is to perform the esterification reaction for production of monoolein in supercritical conditions without the use of organic solvents. In this respect, we report experimental phase equilibrium data for CO_2 + (R,S)-1,2-O-iso-propylidene glycerol (solketal) + Oleic Acid systems. A variable-volume view cell for obtaining the experimental data in the temperature range from 308 to 338 K and pressures up to 20 MPa was used. In the absence of oleic acid a vapor-liquid phase transition as bubble for all compositions investigated was observed. In the ternary system (CO_2 + Solketal + Oleic Acid) the vapor-liquid phase transitions as bubble points was initially observed whereas, at high concentrations of carbon dioxide the transitions were as dew points. It was possible to obtain monoolein using supercritical CO_2, making this technology a promising synthesis method, which can lead to high yields with minimal risk to the environment.

Επίδραση εκχυλισμάτων λευκού και κόκκινου κρασιού στα ενζυμα βιοσύνθεσης του Παράγοντα Ενεργοποίησης Αιμοπεταλίων (PAF)

Effects of red and white wine extracts on PAF biosynthetic enzymes

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Studies support the anti-atherogenic effect of wine. In this concept, our previous results indicate that the polar lipid fractions (**PL**) of a red wine (Cabernet Sauvignon–CS) and of a white one (Robola–R) inhibit PAF-induced platelet aggregation. The lack of data concerning the effect of wine compounds on PAF metabolic enzymes prompted us to investigate this field.

The effect of wine **PL** and **Water (W)** fractions and of resveratrol and quercetin to modulate PAF biosynthetic enzymes, namely Phospholipase A_2 (PLA_2), acetyl-CoA:lyso- PAF acetyltransferase (lyso-PAF-AT) and DTT-insensitive CDP-choline 1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase (PAF-CPT). Thus cell free and cell culture experiments on U937 cell line were performed as well as recombinant PLA_2 was used.

In cell free system resveratrol inhibits both lyso-PAF-AT (IC_{50}= 0.136mg/ml) and PAF-CPT (IC_{50}= 0.0896mg/ml) in a dose-depended manner. Quercetin acts in a non dose-depended manner, but inhibited 50% of lyso-PAF-AT and PAF-CPT activity at 0.2 and 0.3mg/ml respectively. **PL** of both wines inhibit in the same order of magnitude the action of lyso-PAF-AT (IC_{50}:$\mathbf{PL_R}$=1.7, $\mathbf{PL_{CS}}$=1.6mg/ml) and of PAF-CPT (IC_{50}: $\mathbf{PL_R}$=2.91, $\mathbf{PL_{CS}}$=2.8mg/ml). In contrast, $\mathbf{W_{CS}}$ is more effective inhibitor of lyso-PAF-AT and PAF-CPT than $\mathbf{W_R}$ (IC_{50}:$\mathbf{W_R}$=4.2, $\mathbf{W_{CS}}$=0.05mg/ml – $\mathbf{W_R}$=4.6, $\mathbf{W_{CS}}$=1.1mg/ml). All fractions inhibit PLA_2 at the lyso-PAF-AT IC_{50} values at similar percentages ($\mathbf{PL_R}$=53%, $\mathbf{PL_{CS}}$=62%, $\mathbf{W_R}$=62%, $\mathbf{W_{CS}}$=58%).

In cell culture systems resveratrol and quercetin inhibit both enzymes (50% inhibition lyso-PAF-AT: 0.021 and 0.0060mg/ml – PAF-CPT 0.018 and 0.042μg/ml respectively). **PL** fractions are potent inhibitors of both enzymes (50% inhibition at 0.033mg/ml for $\mathbf{PL_R}$ and 0.073mg/ml for $\mathbf{PL_C}$). Concerning **W** fractions seems to posses a dual action since in lower concentrations activate lyso-PAF-AT and PAF-CPT while in higher inhibit only PAF-CPT.

In conclusion, wine compounds could reduce PAF biosynthetic enzymes activity. All compounds exhibit stronger activity in cell culture system indicated a possible modulation of signal transduction pathways instead of a direct action upon enzymes.

ΕΥΡΕΤΗΡΙΟ ΣΥΓΓΡΑΦΕΩΝ