

# OIL TECHNOLOGISTS' ASSOCIATION OF INDIA

JULY 2014 - FEBRUARY 2015

EASTERN REGION



FOR LIMITED CIRCULATION



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**S. K. Roy has taken over as President OTAI & Mr. Janardan as Vice President (H.Q.)  
for the year 2013-15.**

*Editorial Advisory Committee*

D. K. Bhattacharyya, B. V. Mehta  
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*Editor*

S. K. Roy

*For enquiries please contact : S. K. Roy, Editor*

*5C, Tarak Mitra Lane, Kolkata - 700 026, Ph. : 2466 6243 / 2463 9721*

*E-mail : esskay\_roy81@rediffmail.com / es.k.roy@gmail.com*

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## From the Editor's Desk

Fats and other lipids also contribute essential fatty acids to the diet. While it has never been unequivocally demonstrated that these fatty acids are essential to man, the work with eczema in babies indicates that we may have a real need for small amounts of linolenic and arachidonic acids in the diet.

Fats are also solvents of the fat soluble vitamins that are always naturally introduced into the diet in the fatty portion of the food. The fat soluble vitamins are the vitamins A, D, E, and K, and the provitamins A and carotenes. All of these vitamins are, important to optimum health.

The fats and other lipids are, therefore, important in the diet for a number of reasons. Whether or not the amount can exceed optimum is yet to be proved.

Excess of Fat in our diet or the wrong kind of Fat is blamed for various ailments like "Heart Disease", obesity and associated diseases. Our concept of wrong fat is due to their perceived effects on raising cholesterol levels and so contributing to "Heart Disease".

During the past cholestrophobic half century, cholesterol became the most important culprit causing heart disease. Drugs and cholesterol free foods and products were developed to help lower blood cholesterol.

Blaming cholesterol for heart disease became a ready reply like a cause for the effect. The quick relief came in the form of 'Statin Type' Hypocholesterolemic drugs and its abundant overuse.

It may not be out of place to mention that Dr. Jon Kabara, Director R&D, Med, Chem Laboratories Gallena, Illinois, an authority, in this particular discipline published one of the first papers showing that these Hypocholesterolemic drugs at non-toxic dose levels decreased the life span of tumor bearing animals and "Clofibrate", a drug belonging to this category was withdrawn from the market. Another very specific and remarkable observation is the side effects that all statin drugs have on co-enzyme Q10. This-co-enzyme is an important respiratory substance, particularly for heart and skeletal muscle since cholesterol formation needs 6x isopropene units and Q10 needs 10x, it stands to reason that co-enzyme Q10 will be affected more than cholesterol. One effect has been reported from muscle weakness. Recently Global loss of memory has been recorded as another consequence of taking these drugs. These are cosequences, the doctor does not communicate to you, as the medical representative does not pass on this intricate details about the side effects to the doctor.



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All 'Unhealthy' (Saturated) fats are labelled as being bad for our health since they tend to raise cholesterol levels. Unsaturated fatty acids are classified as mono or polyunsaturated (PUFA). The later group is described more precisely as being members of the omega-6 or omega-3 family of fatty acids. At one time Omega-6 fats were recommended as the answer to better health as they seemed to lower cholesterol. But it also came to the notice that n-6 fats were detrimental to immunity, and reproductive system and overshadowed the, positive effects of n-3 fats.

The truth is that not all unsaturated fats are good and not all saturated fats are bad. Dr. Jon Kabara suggests that the term saturated Fat should not be written without the qualifier, short, medium or long chain preceeding the word "saturated". These three groups of fatty acids have completely different physical, biological, pharmacological properties. In fact the medium chain lipid have been used clinically for malabsorption problems for over 25 years and newer studies recently suggests that they also have antimicrobial effects. In addition medium chain fats have fewer calories per gram and are not deposited in adipose tissue as are other fats, both unsaturated and long chain saturated. There is even some disagreement that all long chain saturated fats raise Cholesterol, says Dr. Kabara.

I would therefore request you to be judicious while attempting to derive quick benefit from drugs which are probably associated with delayed damages more detrimental than cure.

Wish you all a very Healthy & Prosperous New Year !!

Wish you all Happy Holi !!

S.K. Roy  
*Editor*

*Acknowledgement*

- Dr. Jon Kabra  
Director, R & D  
Med-Chem Laboratories Inc.  
Galcna, (Illinois)
- Inform-2005
- OTAI News Letter

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## ABOUT OURSELVES

69th Annual Convention and International Conference, was held in AGRA, INDIA, from November 14 to 16, 2014. It was organised by Oil Technologists' Association of India, Central Zone, Kanpur. The conference was well organized and was a grand success. The following members attended the conference.

S.K.Roy, President, OTAI, Chairman, National Organizing Committee, Dr Ranjit Chakrabarty, President, (E.R.), Dr Mahua Ghosh, Hony, Secy Mr T.K.Mitra, C.E.C. Member, Mr Sarjit Singh, Former Hon. Secy.

The details of the participants who took part in presenting research papers in the conference are as follows :

1. Dr. Avery Sengupta, IEST, BESU, Shibpur
  2. Ms. Moumita Pal, IEST, BESU, Shibpur
  3. Ms. Sohini Mukherjee, Dept. of Chem Tech, CU
  4. Ms. Kankana Das, Dept. of Chem Tech, CU
  5. Ms. Soma Das, Dept. of Chem Tech, CU
  1. Comparative study of in vitro digestion and gastrointestinal absorption of medium chain fatty acid rich rice bran and rice bran oil.  
Avery Sengupta<sup>1</sup>, Mahua Ghosh<sup>2</sup> and Dipak Kumar Bhattacharyya<sup>1</sup>.  
<sup>1</sup>School of Community Science and Technology, IEST, Shibpur, Howrah, <sup>2</sup>Dept. of Chemical Technology, University of Calcutta.
  2. Production of medium chain fatty acid (MCFA) containing structured diacylglycerols (DAGs) by enzymatic esterification reaction Kankana Das\* and Mahua Ghosh.  
Dept. of Chemical Technology, University of Calcutta.
  3. Rice bran oil Oleogel: A novel technology for structuring edible oils  
Moumita Ghosh<sup>1</sup>, Mahua Ghosh<sup>2</sup> and Dipak Kumar Bhattacharyya<sup>1</sup>.  
<sup>1</sup>School of Community Science and Technology, IEST, Shibpur, Howrah.  
<sup>2</sup>Dept. of Chemical Technology, University of Calcutta.
  4. PRODUCTION OF MCFA RICH MUSTARD OIL USING PACKED BED BIOREACTOR  
Sohini Mukherjee\*, Susmita Roy and Mahua Ghosh  
Dept. of Chemical Technology, University of Calcutta
  5. Production of Fat Replacer from Mango Starch and its Application in baking.  
Soma Das<sup>1\*</sup>, D. K. Bhattacharyya<sup>2</sup>, Mahua Ghosh<sup>1</sup>  
<sup>1</sup>Dept. of Chemical Technology, University of Calcutta.  
<sup>2</sup>School of Community Science and Technology, IEST, Shibpur, Howrah.  
Two more from BESU, details not known to me.
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## From the Pages of History

### MUSINGS ON NEWS LETTER OF EASTERN REGION OIL TECHNOLOGISTS' ASSOCIATION OF INDIA, CALCUTTA

The news letter of OTAI (EZ) in its present form is being published since 1997; a quarterly publication-at the beginnings under the editorship of Dr.(Mrs.) P. Sengupta and subsequently and at present under the editorship of Mr. S.K. Roy. That the news letter is coming out regularly for the last eight years or so is definitely a matter of pride for us and this is due to dynamic leadership of Mr. Roy and keen interest taken by him. Our hearty congratulations to him and the advisory committee comprising of Dr. R. K. Shah, Dr. D. K. Bhattacharyya, R. S. Vaidyanathan etc who are actively assisting, for their effort.

Each issue of news letter provides its members vast information on different topics of scientific and technological interest through articles on oil and oils based products authored by eminent academicians, scientists, experts and others.

Parliament news and Government notifications are regular features. Book review are published occasionally.

“From President Desk” at the beginning certainly deserves special attention. Each note either focuses our attention on information or stresses new subject areas. To me, the news is a stimulating periodicals and every quarter of a year, I wait for its publication.

At the same time, one shall like to have information on trade and commerce as a regular feature. The news letter may be a discussion forum on scientifically controversial topics.

Additionally, our news letter shall try to incorporate the proceedings or activities of committees or subcommittees of Government or semi-government or NGO organisations that become available from time to time. Special articles on oils and oilseeds published elsewhere may be reproduced. Reporting on Research activity and industrial activity or our country may form regular feature of the news letter.

Dr. D. P. Sen  
*Life Fellow &  
Member, Executive Committee*

Ack :  
May 2005  
E R News Letter

Dr. D. P. Sen has since left for heavenly abode.

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# 13-Oxo-9(Z),11(E),15(Z)-octadecatrienoic Acid Activates Peroxisome Proliferator-Activated Receptor $\gamma$ in Adipocytes

HARUYA TAKAHASHI, HIDEYUKI HARA, TSUYOSHI GOTO, KOSUKE KAMAKARI  
NOMURA WATARU, SHINSUKE MOHRI, NOBUYUKI TAKAHASHI, HIDEYUKI SUZUKI  
DAISUKE SHIBATA, TERUO KAWADA

## ABSTRACT

Peroxisome proliferator-activated receptor (PPAR) $\gamma$  is expressed in adipose tissue and plays a key role in the regulation of adipogenesis. PPAR $\gamma$  activators are known to have potent antihyperglycemic activity and are used to treat insulin resistance associated with diabetes.

Therefore, many natural and synthetic agonists of PPAR $\gamma$  are used in the treatment of glucose disorders. In the present study, we found that 13-oxo-9(Z),11(E),15(Z)-octadecatrienoic acid (13-oxo-OTA), a linolenic acid derivative, is present in the extract of tomato (*Solanum lycopersicum*), Mandarin orange (*Citrus reticulata*), and bitter melon (*Momordica charantia*). We also found that 13-oxo-OTA activated PPAR $\gamma$  and induced the mRNA expression of PPAR $\gamma$  target genes in adipocytes, thereby promoting differentiation.

Furthermore, 13-oxo-OTA induced secretion of adiponectin and stimulated glucose uptake in adipocytes.

To our knowledge, this is the first study to report that 13-oxo-OTA induces adipogenesis through PPAR $\gamma$  activation and to present 13-oxo-OTA as a valuable food-derived compound that may be applied in the management of glucose metabolism disorders.

**Key words :** Keywords PPAR $\gamma$ , Oxylipin, Adipocyte, LC–MS, Adiponectin, Glucose uptake.

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### *Ack/Courtesy :*

H. Takahashi, H. Hara, T. Goto, K. Kamakari, N. Wataru, S. Mohri, N. Takahashi, T. Kawada  
Laboratory of Molecular Functions of Food, Division of Food, Science and Biotechnology, Graduate School of Agriculture,  
Kyoto University, Uji, Kyoto 611-0011.

Japan T. Goto, N. Takahashi, T. Kawada (\*).

Research Unit for Physiological Chemistry, Kyoto University, Kyoto 606-8501, Japan. e-mail: fat@kais.kyoto-u.ac.jp.

H. Suzuki · D. Shibata

Kazusa DNA Research Institute, Kisarazu, Chiba 292-0818, Japan.

Journal Lipids 2015, Vol - 50, Issue - 1, pp 3 to 12.

Springer Berlin, Heidelberg Aocs.

Dr. Kathy Heine - communicated.

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## INTRODUCTION

Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily [1–5]. PPAR are expressed in many tissues including adipocytes, hepatocytes, muscles, and endothelial cells, and play an important role in lipid and glucose homeostasis [6]. The PPAR family comprises three isoforms: PPAR $\alpha$ , PPAR $\beta/\delta$ , and PPAR $\gamma$ . PPAR $\gamma$  is the master regulator of adipocyte differentiation, and functions to enhance the numbers of insulin-sensitive small adipocytes [7]. It is well known that the activation of PPAR $\gamma$  in mature adipocytes regulates several genes involved in glucose metabolism including fatty acid-binding protein (aP2), lipoprotein lipase (LPL), and CCAAT/enhancer binding protein (CEBP)  $\alpha$  [8, 9].

PPAR $\gamma$  and retinoid X receptor (RXR) heterodimers bind lipophilic activators, which induce a conformational change in PPAR $\gamma$  and subsequently regulate gene transcription. The specific DNA regions of PPAR $\gamma$  target genes that bind PPAR are termed PPAR response elements (PPRE) [6]. As such, PPRE are found in the promoters of PPAR $\gamma$ -responsive genes. PPAR $\gamma$  activators, such as thiazolidinediones (TZD), are known to have potent antihyperglycemic activity and are used to treat insulin resistance associated with diabetes [10]. Moreover, recent studies revealed that many compounds derived from food or other natural sources enhance adipocyte differentiation and glucose uptake by activating PPAR $\gamma$  [11–16]. These compounds have PPAR $\gamma$  ligand activity, suggesting that intake of these compounds is valuable for maintaining health.

Many plant oxylipins are formed from unsaturated fatty acids in the lipoxygenase cascade. Oxylipins are known to be potent bioregulators and are involved in the regulation of growth, differentiation, and morphogenesis [17–19]. Recently, it has been reported that various oxylipins isolated from microalgae can inhibit inflammation [20]. Furthermore, our previous studies have revealed that tomatoes contain the oxidized linoleic acid (LIA) derivatives, 9-oxo-10,12-octadecadienoic acid (9-oxo-ODA) and 13-oxo-9,11-octadecadienoic acid (13-oxo-ODA), which serve as PPAR $\alpha$  agonists in mouse liver [21, 22]. Interestingly, previous studies have revealed that 13-oxo-ODA can activate not only PPAR $\alpha$ , [22] but also PPAR $\gamma$  [23].

Plants contain linolenic acid (LNA) as well as LIA, and various oxylipins have also been identified [24]. In this study, we found that 13-oxo-9(Z),11(E),15(Z)-octadecatrienoic acid (13-oxo-OTA) which is an LNA derivative is present in tomato (*Solanum lycopersicum*), Mandarin orange (*Citrus reticulata*), and bitter melon (*Momordica charantia*). Although the structure of 13-oxo-OTA is similar to that of 13-oxo-ODA, little is known about the biofunction involved in the regulation of PPAR $\gamma$  activity.

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We hypothesized that 13-oxo-OTA is a PPAR. activator. Therefore, the aim of the present study was to evaluate the effect of 13-oxo-OTA on PPAR. activity and to elucidate its role in adipocyte differentiation and glucose uptake.

In the present study, we show that 13-oxo-OTA activated PPAR. and induced mRNA expression of PPAR. target genes, which promoted adipocyte differentiation. Furthermore, 13-oxo-OTA induced the secretion of adiponectin and glucose uptake in adipocytes. To the best of our knowledge, this is the first study to report that 13-oxo-OTA induced adipogenesis through PPAR. activation, and the findings suggest that 13-oxo-OTA might be a valuable food-derived compound to improve glucose metabolism disorders.

## **MATERIALS AND METHOD**

### **Materials**

Authentic 13-oxo-ODA and 13-oxo-OTA were purchased from Indofine Chemical Company (Hillsborough, USA) and Laroden Fine Chemicals (Malmö, Sweden), respectively. Authentic *cis*-10-heptadecenoic acid (HDA) was purchased from Sigma (St. Louis, MO). All other chemicals used were from Sigma or Wako (Osaka, Japan), and were guaranteed to be high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC–MS) grade. Tomatoes, Mandarin oranges, and bitter gourds were obtained from the local market.

13-Oxo-OTA Analysis by Ultra Performance Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (UPLC–QToFMS).

Each freeze-dried sample (10 mg) was homogenized with a mixer in 1 mL of extraction solvent (99.5 % EtOH containing 1 µg/mL of HDA). After centrifugation (15,000 rpm, 10 min, 4 °C), the supernatant was collected as an extract. Using an additional 1 mL of extraction solvent, the same procedure was then repeated. The pooled extracts were filtered through a 0.2-µm-pore polyvinylidene difluoride (PVDF) membrane (Whatman, Brentford, UK), and the filtrates were used for LC–MS.

LC–MS was performed using a Waters Acquity UPLC system coupled to a Xevo QTOF-MS (Waters), equipped with an electrospray source, operating in negative ion mode, with a lock-spray interface for accurate mass measurements. The capillary, sampling cone, and extraction cone voltages were set at 2,700, 20, and 1 V respectively. The source and desolvation temperatures were 120 and 450 °C respectively. The mass scan range was set at 50–1,000 *m/z*. The cone and desolvation gas flow rates were set at 50 and 800 L/h respectively, and leucine enkephalin was employed as the lock-mass compound. It was infused straight into the MS at a flow rate of 30 µL/min at a concentration of 200 µg/mL (in 50 % acetonitrile, 50 % water, 0.1 % formic acid). The data were acquired



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with Mass-Lynx software (Waters). External mass calibration was performed following the manufacturer's protocol. An aliquot of the extracted sample (3  $\mu$ L) was injected into an Acquity UPLC BEH-C18 reversed-phase column (column size 2.1  $\times$  100 mm, particle size 1.7  $\mu$ m). Mobile phases A (water and 0.1 % formic acid) and B (acetonitrile and 0.1 % formic acid) were used. The column temperature was set to 40  $^{\circ}$ C. The buffer gradient consisted of 30–50% B for 0–4 min, 50–85 % B for 4–14 min, and was held at 99 % B for 14–17 min and returned to 30 % B for 3 min before the next injection, at a flow rate of 300  $\mu$ L/min.

### **LUCIFERASE ASSAY**

Luciferase assays were performed as previously described, using a GAL4/PPAR chimera system [25, 26]. We transfected p4xUASg-tk-luc (a reporter plasmid), pM-hPPARa (an expression plasmid for a chimera protein of the GAL4 DNA-binding domain and each human PPAR-ligandbinding domain), and pRL-CMV (an internal control for normalizing transfection efficiency) into monkey CV1 kidney cells by using Lipofectamine (Life Technologies Japan Ltd.) according to manufacturer's protocol. Luciferase activity was assayed using the dual luciferase system (Promega, MO, USA) according to the manufacturer's protocol.

### **3T3-L1 ADIPOCYTE CELL CULTURE**

The 3T3-L1 cells were cultured, maintained, and differentiated according to a previously described method [27]. Briefly, 3T3-L1 murine pre-adipocytes were cultured in growth medium (Dulbecco's modified Eagle's medium [DMEM] supplemented with 10 % (v/v) fetal bovine serum, 100 units/mL penicillin, and 100  $\mu$ g/mL streptomycin) at 37 $^{\circ}$ C in 5% CO<sub>2</sub>. At 2 days after reaching confluence, the cells were incubated in a differentiation medium (containing 0.25  $\mu$ M dexamethasone, 10  $\mu$ g/ml insulin, and 0.5 mM 1-methyl-3-isobutylxanthine [IBMX]) for 48 h and then in growth medium containing 5  $\mu$ g/ml insulin for an additional 2 days. On the 4th day, this was replaced with fresh growth medium. 13-Oxo-OTA, 13-oxo-ODA, LNA, LIA, or troglitazone were treated from day 0 to day 6. Total adiponectin was determined using the Mouse Adiponectin ELISA kit (R&D Systems, Minneapolis, MN). Oil-Red O staining was performed as described previously [28]. Quantification of triglyceride (TG) using the TG E-test Kit (Wako) was performed as described previously [29].

### **QUANTIFICATION OF MRNA EXPRESSION LEVELS**

Total RNA was prepared from 3T3-L1 cells by using Sepasol (Nacalai Tesque), according to the manufacturer's protocols. Using M-MLV reverse transcriptase (Life Technologies Japan Ltd.), total RNA was reverse-transcribed using a thermal cycler (Takara PCR

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Thermal Cycler SP: Takara BioInc., Shiga, Japan). To determine mRNA expression levels, real-time quantitative RT-PCR analysis was performed with a Light Cycler System (Roche Diagnostics) using SYBR green fluorescence signals as described previously [25, 30]. The oligonucleotide primer sets of mouse 36B4 and PPAR. target genes were designed using a PCR primer selection program at the website of the Virtual Genomic Center from the GenBank database as follows: mouse PPAR. (Fwd: 5'-ggagatctccagtgatatcgacca-3'; Rev: 5'-acggcttctacggatcgaaact-3'), mouse aP2 (Fwd: 5'-aagacagctcctcctcgaagggtt-3'; Rev: 5'-tgaccaaatacccatctacgc-3'), mouse LPL (Fwd: 5'-atccatggatggacggtaacg-3'; Rev: 5'-ctggattccaatacttcgacca-3'), mouse CEBPa (Fwd: 5'-tggacaagaacagcaacgag-3'; Rev: 5'-tcactggtaactccagcac-3'), and mouse 36B4 as an internal control (Fwd: 5'-tccttctccaggcttggg-3'; Rev: 5'-gacaccctccagaaagcgag-3'). All data indicating mRNA expression levels are presented as a ratio relative to a control in each experiment.

### **GLUCOSE UPTAKE INTO 3T3-L1 ADIPOCYTES**

2-Deoxy-d-[3H] glucose (Amersham Biosciences, Piscataway, NJ, USA) uptake was assayed as described previously with modifications [31]. Briefly, prior to the assay, the cells were deprived of serum for 18 h. The cells were then incubated in HEPES-Krebs-Ringer (HKR) buffer (with 0.1 % BSA) in the presence or absence of 100 nM insulin for 20 min at 37 °C. At the end of the incubation period, 2-deoxy-d-[3H] glucose was added to a final concentration of 0.5 µCi/mL. After incubation for 10 min, the reaction was terminated by three washes of ice-cold phosphatebuffered saline (PBS). The cells were solubilized in 0.1 N NaOH, and aliquots of the cell lysate were transferred to scintillation vials for radioactivity counting. Non-specific deoxyglucose uptake was measured in the presence of 10 µM cytochalasin B.

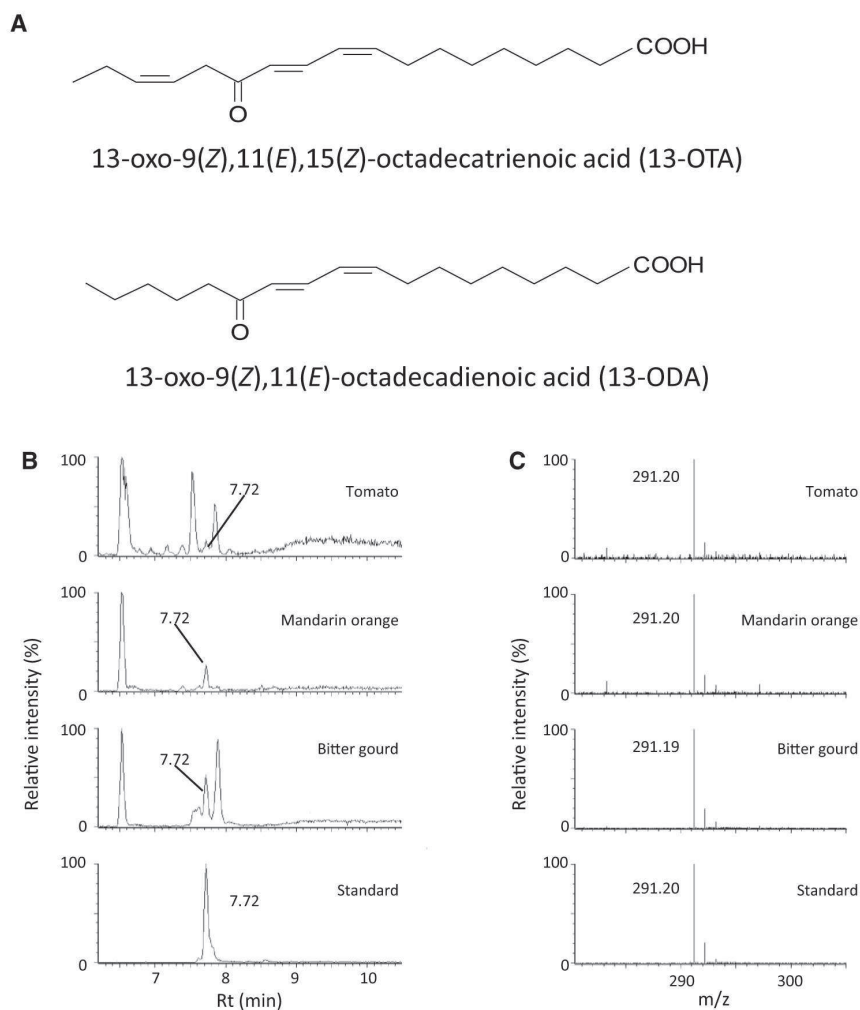
The remainder of the lysate was used for measuring protein content with a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). The radioactivity measurements were normalized by protein concentrations.

### **IMMUNOBLOTTING**

Total cellular proteins were solubilized in lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.5 % deoxycholate, 0.1 % sodium dodecyl sulfate [SDS]; pH 7.4) containing a protease inhibitor cocktail. The protein concentration of samples was determined using a protein assay kit (Bio-Rad Laboratories). Protein samples (20 µg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 5% gel. Separated proteins were transferred electrophoretically to PVDF membranes (Millipore, MA), which were blocked with 5% nonfat dried milk in PBS. The membranes were incubated with antibodies to PPAR $\alpha$  (Cell Signaling Technology, MA) and  $\beta$ -actin



(Cell Signaling Technology, MA), and then with peroxidase-conjugated anti-mouse and antirabbit IgG antibodies (Santa Cruz, CA), respectively. To study adiponectin isoforms, we performed western blotting with Mini-PROTEAN® TGX™ Gels 4–15 % (BIO RAD, Tokyo, Japan) and used an antibody against adiponectin (Affinity BioReagents™, Golden, CO). Proteins were detected using an ECL western blotting detection system (GE Healthcare, NJ).



**Fig. 1** Identification of 13-oxooctadecatrienoic acid (13-oxo- OTA) by LC–MS. a The structure of 13-oxo-OTA and 13-oxo-ODA. b Extracted ion chromatogram ( $m/z = 291.20$ ) data and c full mass data of 13-oxo-OTA in authentic sample and plant extraction sample.

The elution peak ( $R_t = 7.7$  min,  $m/z = 291.20$ ) is 13-oxo-OTA.

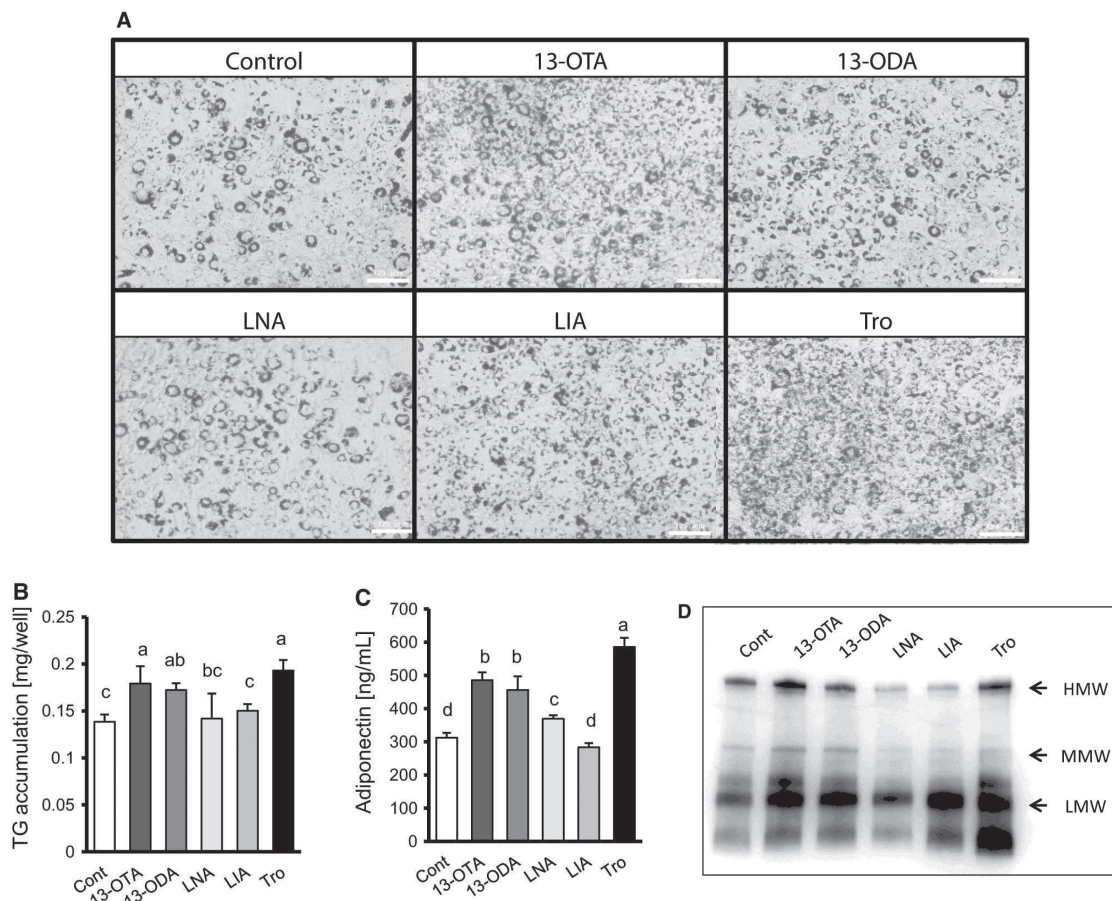
## STATISTICAL ANALYSIS

Data are presented as means  $\pm$  SEM. Differences between groups were determined with one-way analysis of variance (ANOVA), followed by Duncan's test. Values of  $p < 0.05$  were considered statistically significant.

## RESULTS

### Identification of 13-Oxo-OTA in Tomato, Mandarin Orange, and Bitter Gourd Extraction.

We first explored the oxylipins induced LNA from plants extract using an LC–MS system. LC–MS was performed to reveal that 13-oxo-9(Z),11(E),15(Z)-octadecatrienoic acid (13-oxo-OTA, Fig. 1a), which is analogous to 13-oxo-9(Z),11(E)-octadecadienoic acid (13-oxo-ODA, Fig. 1a), was contained in the extract of tomato (*Solanum lycopersicum*), Mandarin orange (*Citrus reticulata*), and bitter gourd (*Momordica charantia*) ( $R_t = 7.72$  min,  $m/z = 291.20$ , Fig. 1b, c). The peak of 13-oxo-OTA was detected as unfragmented deprotonationion ( $C_{18}H_{27}O_3^-$ ,  $[M-H]^-$ , Fig. 1c) under conditions of electrospray negative ionization mass spectrometry.



**Fig. 2** 13-Oxo-OTA promoted adipogenesis and induced the secretion of adiponectine in 3T3-L1 adipocytes. a, b lipid accumulation and the secretion of c total-adiponectin or d HMW-adiponectin in 3T3-L1 adipocytes treated with 13-oxo-OTA, 13-oxo-ODA, LNA, LIA (10  $\mu$ M), or Tro (5  $\mu$ M). Data are presented as the mean  $\pm$  SEM ( $n = 3-5$ ). \* $p < 0.05$ , \*\* $p < 0.01$  vs. Cont. Magnification is  $\times 200$  (white bar is 125  $\mu$ m). Cont Control, Tro troglitazone, LNA linolenic acid, LIA linoleic acid.

### 13-Oxo-OTA Promoted TG Accumulation and Induced Secretion of Adiponectin in Adipocytes.

To investigate the effects of 13-oxo-OTA, 13-oxo-ODA, LNA, and LIA on triglyceride (TG) accumulation, 3T3-L1 adipocytes were cultured in a medium containing these compounds. Oil Red-O staining revealed that lipid accumulation in 3T3-L1 adipocytes increased following 13-oxo-OTA treatment compared to that observed with LNA and LIA (approximately 1.3-fold, Fig. 2a, b). We also investigated the effect of 13-oxo-OTA on secretion of adiponectin in 3T3-L1 adipocytes and found that secretion of total adiponectin increased because of 13-oxo-OTA treatment (approximately 1.6-fold, Fig. 2c). In particular, 13-oxo-OTA increased the secretion of high-molecular-weight (HMW) adiponectin (Fig. 2d). These data suggest that 13-oxo-OTA promotes TG accumulation and induces adiponectin secretion in 3T3-L1 adipocytes.

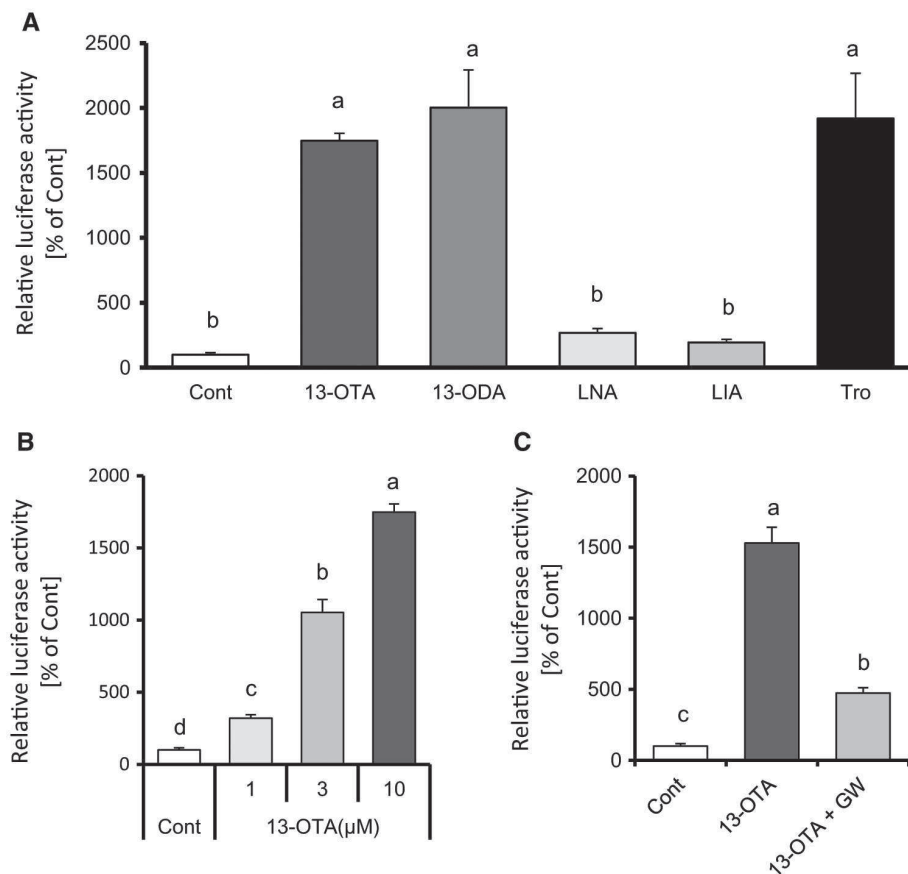
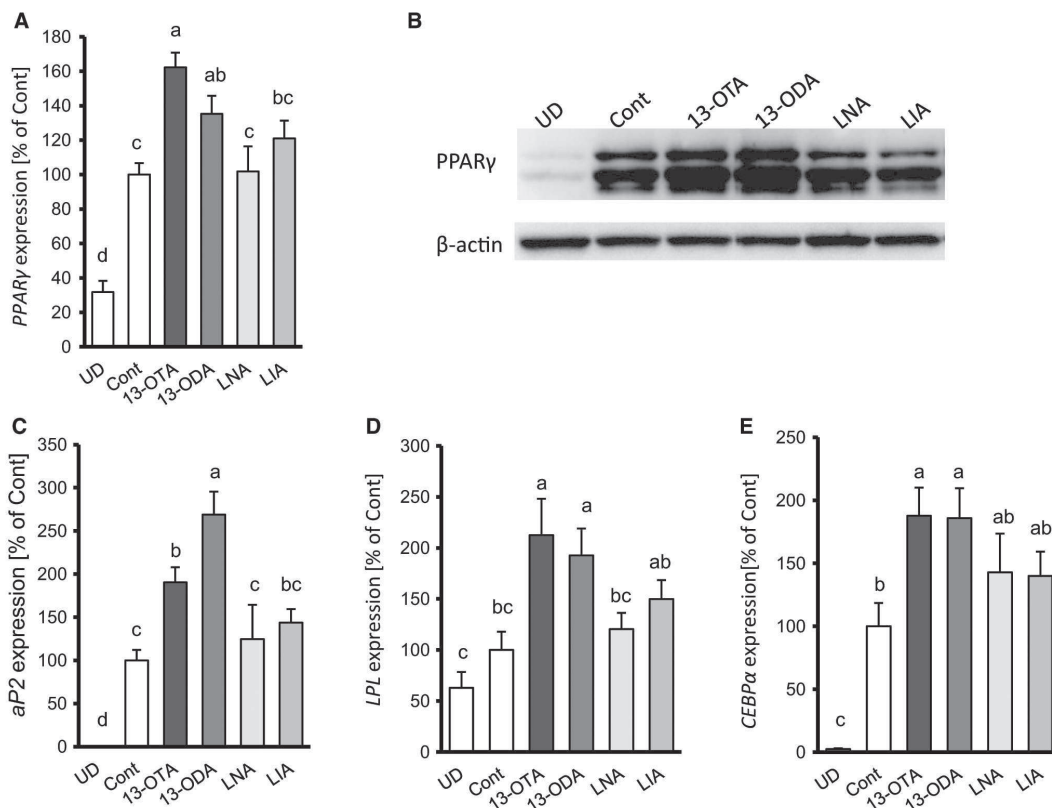


Fig. 3 13-Oxo-OTA activated PPAR $\alpha$ . a Evaluation of PPAR $\alpha$  activity in luciferase reporter assay treated with 13-oxo-OTA, 13-oxo-ODA, LNA, LIA (10  $\mu$ M respectively), or Tro (5  $\mu$ M). b The dose-dependent effect of 13-oxo-OTA on PPAR $\alpha$  activity. c evaluation of PPAR $\alpha$  activity in luciferase reporter assay treated with 13-oxo-OTA and/or PPAR $\alpha$  antagonist (10  $\mu$ M respectively). Data are mean  $\pm$  SEM (n = 5). Cont Control, Tro troglitazone, LNA linolenic acid, LIA linoleic acid, GW GW9662.

### 13-Oxo-OTA Activated PPAR $\alpha$ and Induced mRNA Expression of PPAR $\alpha$ Target Genes

PPAR $\alpha$  is the master regulator of adipocytes differentiation [7]. To elucidate the contribution of 13-oxo-OTA to PPAR $\alpha$  activation, we investigated whether 13-oxo-OTA activated PPAR $\alpha$  in a luciferase ligand assay. 13-Oxo-OTA and 13-oxo-ODA increased luciferase activity remarkably compared to their precursors, LNA and LIA, respectively (Fig. 3a). This activity was increased in a concentration-dependent manner by 13-oxo-OTA treatment (Fig. 3b) and inhibited by PPAR $\alpha$  antagonist (GW9662) treatment (Fig. 3c). In 3T3-L1 adipocytes, the expression level of PPAR $\alpha$  was increased by 13-oxo-OTA treatment (approximately 1.6-fold, Fig. 4a). In addition to increased mRNA levels, up-regulation of PPAR $\alpha$  protein levels was observed (Fig. 4b). Furthermore, mRNA expression levels of PPAR $\alpha$  target genes, such as aP2, LPL, and CEBP $\alpha$ , were increased in 3T3-L1 adipocytes because of treatment with 13-OTA (approximately 1.9-, 2.1-, and 1.9-fold respectively, Fig. 4c– e). These findings suggest that 13-OTA promotes adipocyte differentiation via a PPAR $\alpha$ -dependent pathway.

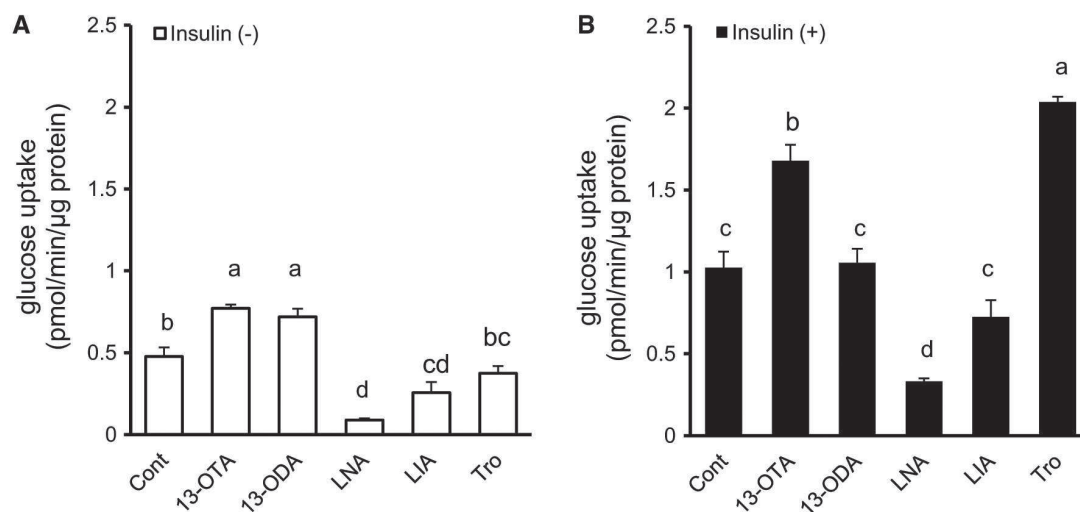


**Fig. 4** 13-Oxo-OTA activated PPAR $\alpha$  target genes expression in 3T3-L1 adipocytes. Effect of 13-oxo-OTA (10 iM) on a PPAR $\alpha$  gene expression and b protein level. Effect of 13-oxo-OTA on c aP2, d CEBP $\alpha$ , and e LPL, PPAR $\alpha$  target genes expression. Data are presented as means  $\pm$  SEM (n = 3–5). \*p < 0.05, \*\*p < 0.01 vs. Cont. UD undifferentiated 3T3-L1 cells.

To elucidate the contribution of 13-oxo-OTA to glucose uptake, we investigated whether 13-oxo-OTA promotes glucose uptake in a radio isotopic assay. We demonstrate here that 13-oxo-OTA promoted glucose uptake in 3T3-L1 adipocytes treated with (approximately + 0.9 pmol/min/ $\mu$ g protein compared with control, Fig. 5b) or without (approximately + 0.3 pmol/min/ $\mu$ g protein compared with control, Fig. 5a) insulin. As compared with 13-oxo-ODA, 13-oxo-OTA has an intense effect on glucose uptake in adipocytes treated with insulin (13-oxo-OTA: approximately 1.6 pmol/min/ $\mu$ g protein, 13-oxo-ODA: approximately 1.0 pmol/min/ $\mu$ g protein, Fig. 5b). Although 13-oxo-OTA stimulated glucose uptake, LNA and LIA inhibited this phenomenon in 3T3-L1 adipocytes treated with or without insulin (Fig. 5a, b). These findings suggest that 13-oxo-OTA stimulates glucose uptake in 3T3-L1 adipocytes.

## DISCUSSION

In the present study, the analysis of metabolites in plant extracts by using LC–MS revealed that 13-oxo-OTA in extracts of tomato, Mandarin orange, and bitter gourd. 13-Oxo-OTA is an LNA derivative. We confirmed that LNA was present in these plants as well (data not shown). It is proposed that 13-oxo-OTA can be derived from LNA via a non-enzymatic and/or enzymatic reaction. It is possible that LNA is susceptible to oxidation at C13 by an auto-oxidation reaction and/or that the activity of 13-lipoxygenase is involved in the production of 13-oxo-OTA in these fruits.



**Fig. 5** 13-Oxo-OTA enhanced glucose uptake in 3T3-L1 adipocytes. Effect of 13-oxo-OTA, 13-oxo-ODA, LNA, LIA (10  $\mu$ M) or Tro (5  $\mu$ M) on glucose uptake in 3T3-L1 adipocytes treated with or without insulin. Data are presented as the mean  $\pm$  SEM ( $n = 3-5$ ). \* $p < 0.05$ , \*\* $p < 0.01$  vs. Cont. INS: insulin (100 nM).



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PPAR $\alpha$  is expressed principally in adipose tissue and plays a key role in the regulation of adipogenesis, energy balance, and lipid biosynthesis [9, 32–34]. Many natural and synthetic agonists of PPAR $\alpha$  are used in the treatment of glucose disorders [6]. Although previous studies have reported that 13-oxo-ODA derived from LIA contributes to activate PPAR $\alpha$  [23], little is known about the effect of 13-oxo-OTA on PPAR $\alpha$ . The structure of 13-oxo-OTA is similar to that of 13-oxo-ODA, which is derived from LIA. We therefore hypothesized that 13-oxo-OTA also has an ability to activate PPAR $\alpha$ .

We demonstrated for the first time that 13-oxo-OTA activates PPAR $\alpha$  and contributes to increased mRNA expression of PPAR $\alpha$  target genes including aP2, LPL, and CEBP $\alpha$ , which are differentiation markers in adipocytes [35]. These findings suggested that 13-oxo-OTA promotes differentiation in 3T3-L1 adipocytes via a PPAR $\alpha$ -dependent pathway. Oil Red-O staining definitively demonstrated that lipid accumulation in 3T3-L1 adipocytes increased owing to 13-oxo-OTA treatment, corroborating our observations following analysis of gene expression. Although 13-oxo-OTA and 13-oxo-ODA both activated PPAR $\alpha$ , LIA and LNA had little effect on PPAR $\alpha$ , suggesting that the keto group (C13 position) plays an important role in binding to PPAR $\alpha$ . In a previous study, it was demonstrated that LIA and LNA have no effect on the expression of PPAR $\alpha$  [36]. Our data confirm the finding from this previous study.

Our present study also showed that 13-OTA induced the secretion of adiponectin and promoted glucose uptake in 3T3-L1 adipocytes. PPARE exists in the adiponectin promoter region [37]. PPAR $\alpha$  agonists therefore stimulate the release of adiponectin from adipocytes. Adiponectin stimulates fatty acid oxidation and contributes to improving glucose metabolism disorders, including insulin resistance [38]. In the present study, 13-oxo-OTA increased the secretion of high-molecular-weight (HMW) adiponectin. Recently, several studies have designated adiponectin isoforms as low (LMW)- or HMW-adiponectin [39]. It has also been suggested that the treatment of diabetic subjects with TZD (PPAR $\alpha$  ligands) specifically increases blood levels of the HMW-adiponectin, and HMW-adiponectin correlates strongly with increased insulin sensitivity [39, 40]. This evidence suggests that HMW-adiponectin induced by 13-OTA treatment contributes to an improvement in glucose metabolism disorders. Indeed, our findings demonstrate that 13-oxo-OTA stimulates glucose uptake in 3T3-L1 adipocytes.

Interestingly, 13-oxo-OTA promotes glucose uptake in 3T3-L1 adipocytes treated with or without insulin, suggesting that 13-oxo-OTA is capable of enhancing glucose uptake via both insulin-dependent and -independent pathways. In a previous study, it was reported that coyote melon (*Ibervillea sonora*) extract induces glucose uptake in adipocytes without insulin and that this effect is exerted by activation of a PI3 K-independent pathway

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[41]. This finding raises the possibility that the effect of 13-oxo-OTA is also exerted by a similar mechanism. The preceding study also revealed that the oxidative stress in adipocytes might be the origin of obesity [42]. Oxidative stress in adipocytes impaired insulin signals and decreased insulin-stimulating glucose uptake [43]. Our data showed the possibility that 13-oxo-OTA is effective in improving oxidative stress in adipocytes. Although 13-oxo-OTA and 13-oxo-ODA contributed to the stimulation of glucose uptake, LIA and LNA inhibited glucose uptake in adipocytes, with or without insulin. Further examination is necessary to determine the mechanism of glucose uptake in adipocytes treated with 13-oxo-OTA or LNA.

Although our present study reveals that 13-oxo-OTA is involved in PPAR $\alpha$  activation in vitro, little is known about 13-oxo-OTA bioactivation in vivo. It is expected that 13-oxo-OTA contained in food (exogenous 13-oxo-OTA) effects adipocytes throughout the bloodstream. 13-Oxo-OTA is one of the oxylipins that have various patterns including fatty acid hydroperoxides, hydroxyl-, oxo-, epoxy, or keto fatty acids, divinyl ethers, aldehydes, or jasmonic acid [17]. Oxylipins are widespread in nature, occurring in plants, animals, mosses, algae, bacteria, and fungi [20]. Indeed, several oxygenated derivative of fatty acids in animal have been found to be biological activity [44, 45]. In a previous study, it was demonstrated that nitroalkene derivatives of LIA serves as an endogenous PPAR $\alpha$  ligand [46]. These findings raise the possibility that not only exogenous, but also endogenous 13-oxo-OTA, is involved in bioactivity in animals. Therefore, it is necessary to evaluate oxylipins in vivo to investigate the effect of both exogenous and endogenous oxylipins.

In conclusion, 13-oxo-OTA contained in tomato, Mandarin orange, and bitter gourd activates PPAR $\alpha$  and induces mRNA expression of PPAR $\alpha$  target genes. These, in turn, promote differentiation and induce secretion of adiponectin and glucose uptake in 3T3-L1 adipocytes. These findings provide the first evidence that 13-oxo-OTA induces adipogenesis through a PPAR $\alpha$ -dependent pathway and contributes to improving treatments for glucose metabolism disorder. These data suggest that 13-oxo-OTA might be a valuable, food-derived compound for maintaining health.

### **Acknowledgments**

The authors thank S. Shinoto and M. Sakai for secretarial and technical support, respectively. This work was supported in part by Research and Development Projects for Application in Promoting New Policies Agriculture, Forestry, and Fisheries of Japan, by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (22228001 and 24688015), and by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (24521).

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## REFERENCES

1. Escher P, Wahli W (2000) Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat Res* 448:121–138.
2. Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 20:649–688.
3. Chinetti G, Fruchart JC, Staeles B (2000) Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res* 49:497–505.
4. Duval C, Chinetti G, Trottein F, Fruchart JC, Staeles B (2002) The role of PPARs in atherosclerosis. *Trends Mol Med* 8:422–430.
5. Goldenberg I, Benderly M, Goldbourt U (2008) Update on the use of fibrates: focus on bezafibrate. *Vasc Health Risk Manag* 4:131–141.
6. Grygiel-Górniak B (2014) Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications—a review. *Nutr J* 13:17.
7. Brun RP, Spiegelman BM (1997) PPAR gamma and molecular control of adipogenesis. *J Endocrinol* 155:217–218.
8. Kintscher U, Law RE (2005) PPARgamma-mediated insulin sensitization: the importance of fat versus muscle. *Am J Physiol Endocrinol Metab* 288:E287–E291.
9. Lehrke M, Lazar MA (2005) The many faces of PPARgamma. *Cell* 123:993–999.
10. Nolan JJ, Ludvik B, Beedsen P, Joyce M, Olefsky J (1994) Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Engl J Med* 331:1188–1193.
11. Han KL, Jung MH, Sohn JH, Hwang JK (2006) Ginsenoside 20(S)-protopanaxatriol (PPT) activates peroxisome proliferator-activated receptor . (PPAR.) in 3T3-L1 adipocytes. *Bio Pharm Bull* 29:110–113.
12. Yang Y, Shang W, Zhou L, Jiang B, Jin H, Chen M (2007) Emodin with PPAR. ligand-binding activity promotes adipocyte differentiation and increases glucose uptake in 3T3-L1 cells. *Biochem Biophys Res Commun* 353:225–230.
13. Shang W, Yang Y, Jiang B, Jin H, Zhou L, Liu S, Chen M (2007) Ginsenoside Rb1 promotes adipogenesis in 3T3-L1 cells by enhancing PPAR.2 and C/EBPα gene expression. *Life Sci* 80:618–625.
14. Choi SS, Cha BY, Lee YS, Yonezawa T, Teruya T, Nagai K, Woo JT (2009) Magnolol enhances adipocyte differentiation and glucose uptake in 3T3-L1 cells. *Life Sci* 84:908–914.
15. Shin DW, Kim SN, Lee SM, Lee W, Song MJ, Park SM, Lee TR, Baik JH, Kim HK, Hong JH, Noh M (2009) (-)-Catechin promotes adipocyte differentiation in human bone marrow mesenchymal stem cells through PPAR gamma transactivation. *Biochem Pharmacol* 77:125–133.



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16. Takahashi N, Yao R, Kang MS, Senda M, Ando C, Nishimura K, Goto T, Hirai S, Ezaki Y, Kawada T (2011) Dehydroabietic acid activates peroxisome proliferator-activated receptor- and stimulates insulin-dependent glucose uptake into 3T3-L1 adipocytes. *BioFactors* 37:309–314.
  17. Grechkin A (1998) Recent developments in biochemistry of the plant lipoxygenase pathway. *Prog Lipid Res* 37:317–352.
  18. Farmer EE, Almeras E, Krishnamurthy V (2003) Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Curr Opin Plant Biol* 6:372–378.
  19. Gardner AN, Tarchevsky IA (1999) The lipoxygenase signaling system. *Russ J Plant Physiol* 46:114–123.
  20. Reyes C, Ávila-Román J, Ortega MJ, Jara A, García-Mauriño S, Motilva V, Zubía E (2014) Oxylipins from the microalgae *Chlamydomonas debaryana* and *Nannochloropsis gaditana* and their activity as TNF- $\alpha$  inhibitors. *Phytochemistry* 102:152–161
  21. Kim YI, Hirai S, Takahashi H, Goto T, Ohyan C, Tsugane T, Konishi C, Fujii T, Inai S, Iijima Y, Aoki K, Shibata D, Takahashi N, Kawada T (2011) 9-oxo-10(E),12(E)-Octadecadienoic acid derived from tomato is a potent PPAR  $\alpha$  agonist to decrease triglyceride accumulation in mouse primary hepatocytes. *Mol Nutr Food Res* 55:585–593.
  22. Kim YI, Hirai S, Goto T, Ohyan C, Takahashi H, Tsugane T, Konishi C, Fujii T, Inai S, Iijima Y, Aoki K, Shibata D, Takahashi N, Kawada T (2012) Potent PPAR $\alpha$  activator derived from tomato juice, 13-oxo-9,11-octadecadienoic acid, decreases plasma and hepatic triglyceride in obese diabetic mice. *PLoS One* 7:e31317.
  23. Reinhold A, Martin H, Tanja S, Michael G, Arthur WB, Katrin M, Daniela V, Hans H, Jürgen S, Werner F, Gerhard R (2007) 13-Oxo-ODE is an endogenous ligand for PPAR. in human colonic epithelial cells. *Biochem Pharmacol* 74:612–622.
  24. Smilowitz JT, Zivkovic AM, Wan YJ, Watkins SM, Nording ML, Hammock BD, German JB (2013) Nutritional lipidomics: molecular metabolism, analytics, and diagnostics. *Mol Nutr Food Res* 57:1319–1335.
  25. Takahashi N, Kawada T, Goto T, Yamamoto T, Taimatsu A, Matsui N, Kimura K, Saito M, Hosokawa M, Miyashita K, Fushiki T (2002) Dual action of isoprenols from herbal medicines on both PPAR. and PPAR $\alpha$  in 3T3-L1 adipocytes and HepG2 hepatocytes. *FEBS Lett* 514:315–322.
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# **ALOE VERA (*ALOE BARBADENSIS MILLER*) CHARACTERIZATION AND APPLICATION**

by

**CLAUDIA COBZARU<sup>1\*</sup>, CORINA CERNĂTESCU<sup>1</sup>  
and ADRIANA MARINOIU<sup>2</sup>**

## **ABSTRACT**

In recent decades the medical investigations have confirmed and extended the range of the curative properties attributable to the “biter substance” in the plant Aloe Vera. Although it is not the only species with curative effects Aloe Vera gives the best results as settled by the most recent investigations in the field. The most valuable component of the plant is the gel containing more than 75 nutrient substances, 200 active components, 20 minerals, 18 amino acids and 12 vitamins. Due to its complex composition, the Aloe Vera gel could to be found in the composition of many cosmetics for its hydrating, emollient, anti-inflammatory effects with no irritating action on the skin.

A large number of studies on the chemical composition of the plant and the uses in medicine and cosmetic fields are reported in literature and their analysis is the aim of the present paper.

**Key words :** Aloe Vera, chemical composition, extraction, applications.

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*Ack :*

<sup>1</sup>“Gheorghe Asachi” Technical University of Iași,  
Faculty of Chemical Engineering and Environmental Protection

<sup>2</sup>National R&D Institute for Cryogenics and Isotopic Technologies-ICIT, Râmnicu Vâlcea

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## INTRODUCTION

Aloe Vera (*Aloe Barbadensis Miller*) is a subtropical plant of the *Liliaceae* species known for countries as the medical plant due to its extraordinary therapeutic effects. The name of “aloe” comes from the Arabic word “aloeh” meaning in translation “crystalline and bitter sap”. The name of *Aloe Barbadensis Miller* was given in honor of the botanist Miller who was the first to describe the plant Aloe in the Barbados islands (Youngken, 2000).

The leaves of this plant are pulpous, lance shaped and the edges are tooth shaped. Since it grows in the subtropical regions it cannot survive in other places where the temperature decreases below zero degrees for too long. Consequently, Aloe Vera is naturally encountered in the Northern, Eastern and Southern Africa, in the countries around Mediterranean Sea, Red Sea, in India, China and the islands in the Indian Ocean.

On the other hand, it is also cultivated especially in the islands in Caribbean Sea, Dominican Republic, Barbados, Jamaica and Puerto-Rico. The cultivated plant is 75-120 cm height, has 12-15 wide and pulpous leaves weighting between 700-1500 g each. The leaf size depends on the soil quality and weather conditions. The plant is mature in 3-4 years and this is the proper time for harvesting (Adushan, 2008). Large areas cultivated with Aloe are found in Texas, California, Arizona and Mexic. More than 250 species of Aloe grow in the world such as: Aloe Vera Linné, Aloe Vera Lemarck etc. (Tucker *et al.*, 1989)

The therapeutic effects of the plant have been known since ancient times. A stone plate found in an Egyptian grave dating 3500 years ago is taken as the first document mentioning the Aloe plant. The Egyptians of that times referred to this plant as “the immortality plant” and used to place it among the funeral objects buried in the Pharaoh’s grave for the sake of his spiritual health after death. Moreover, hundred years ago the Africans used to hang up bunches of the plant above the door to get away the bad spirits (Cojan, 2012). Another medical treatise entitled “Papyrus Ebers” written about between 1553-1550 B.C. describes the curing effect of Aloe Vera (Bruneton, 1995; Hänsel, 1994).

The plant Aloe Vera is important for the following two components: the Aloine-an yellow compound that has been using for many years as a laxative ingredient in the pharmaceutical industry, and the *Aloe gel* – a semisolid substances containing amino acids, vitamins, enzymes, proteins, polysaccharides etc, used in phytotherapy (as a basic ingredient in nutritional drinks) and in cosmetic industry (Bruneton, 1995).

## CULTIVATION, GATHERING AND STORING

The studies on the cultivation, gathering and processing the plant are rather scarcely reported in literature the only mention being commonly made that these operations can be performed depending on the plant species, growing region and climate.

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Speaking about the flower pot plant, the cuttings are firstly kept for a few days in a sandy soil under heating and shadow conditions till the roots occur and then put into a flower pot containing garden soil and a quit low amount of fertilizer. Due to its tropical origin Aloe Vera requires rather higher temperatures since the lower ones, such as 4-5°C, destroy the active components in the leaves.

In winter time the plant must be kept inside, in a well aerated place, at a temperature not lower than 10°C. In spring it can be taken out in the garden or in balcony but not overnight when the temperatures are still low. Aloe Vera is not a pretentious plant, it requires to be periodically wetted and a well drained soil. Moreover, the soil must be completely dried when the plant is wetted especially in winter time when more moisture is retained. The gathering is only handmaid and only the mature plants (4-5 years) are collected. Every 2 months 3-5 leaves of larger size can be collected by detaching them just under the line marking the internal gel. Gel gathering operation begins with an incision made on the leaf for taking out a small amount of the translucent and semisolid gel, then by the leaf peeling the entire gel amount is released (Hänsel, 1994; Newton, 1979).

The cultivation of Aloe Vera by the mechanized equipments requires cuttings at intervals of 60-70 cm. The gathering is made both by hand or mechanically under the optimum conditions. In this connection the leaves are cut separately and spun, the gel separated from the leaf peel and subsequently filtered and immediately sterilized by pasteurizing. The contained water is partially removed by reverse osmosis at the temperature of 15°C (Newton, 1979).

The concentrated gel thus obtained is further processed as a powder concentrated by lyophilizing, drying by atomization or cryogenic methods. For instance, the Aloe powder obtained by cryogenic methods contains more active components of the original gel than the lyophilizing products. Furthermore, the process is more efficient, requires a shorter drying time and can be optimized while the final products are of a higher quality (Terry Laboratories, 2012).

## **CHEMICAL COMPOSITION**

The chemical composition of Aloe Vera is very rich in various compounds such as anthraquinone compounds, amino acids, sterols, hormones, vitamins, minerals, enzymes, saponins, lignins and polysaccharides in variable contents in the different plant component parts (Lee & Weintraub, 2000).

*Aloin* is about 40-50% of the plant. It is sparingly water soluble and can be separated by means of oxidizing agents only. Bozzi *et al.* (2006) made evident that the plant peel contains more aloin than the gel. A through study on the therapeutic effect of this plant reported by Singh *et al.* (2010) revealed the Aloe plant to be the best remedy against skin burns as well as the best basic ingredients of pharmaceuticals used as laxatives or against fatness. However, Adushan (2008) has pointed out that the aloin acts as an

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antioxidant with negative effects when used excessively. Wang *et al.* (2011) made a comparison between the following three extraction methods: extraction assisted by microwaves, extraction with ultrasounds and extraction in a Soxhlet device. The allantoin amount resulting by these variants was estimated by HPLC. The experimental results afforded the conclusion that the first two methods are much more rapid, require a lower solvent amount and afford significant extract amount.

The GCMS analysis carried out by Narsih *et al.* (2012) on the peel extract of Aloe Vera identified more active components: squalen (23.60%), 7-tetradecan (19.66%), limonen (13.86%), n-hexadecanoic acid (10.20%), campesterol (1.60%),  $\beta$ -sitosterol (1.37%), methyl 9-octadecanoic ester (7.56%), carvon (9.44%), comaric (7.64%), lupeol (3.45%) and eicosan (1.58%). Apart from this, several functional groups and compound types were identified by the FTIR analysis of the same sample, such as NO<sub>2</sub> nitro group, Ar-OH phenolic, substituted alkenes, eters R-OR, secondary alcohols, R-OH, carboxylic acids RCOOH (Alupului *et al.*, 2007). As found by Narsih *et al.*, (2012) the extract obtained from the Aloe Vera peel at 80°C and 60 min shows a high antioxidant activity. A similar ascertainment belongs to Miladi & Damak (2008) ad Lakshmi & Pa Rajalakshami (2011) that proved the antioxidant properties of the peel to be attributable to the volatile components: tetradecanoic acid, methyl hexadecanoic ester, n-hexadecanoic acid etc.

*The Aloe gel* contains about 0.5% solid components. The gel is extracted from the leaves and has a pH value between 4-5 depending on the climate, season and region. It contains generally more than 75 nutrients, 200 active components, 20 minerals, 18 amino-acids and 12 vitamins. However, the fresh gel is not much used since it is fast oxidized in air and thus the most part of active principles are destroyed in about two hours.

Among the amino acids the glutamic, malic, succinic, citric acids, histidine, lysine, phenylalanine, cystine, methionine, threonine, leucine and valine are mentioned. The concentrations of the malic and citric acids were found to depend on the storage place of the leaves (in dark or at light). Other components, such as quinone and anthraquinone derivatives as well as six enzymes (cellulase, catalase, amilase, carboxypeptidase, oxidase and kinase) were also identified as gel components. Among the identified sugars are : the manose, glucose, low amounts of xilose, arabinose, galactose and ramnose (Meadows, 1980; Zont *et al.*, 2000; Tvet, 1903). The gel obtained from Aloe Vera shows some characteristic actions such as: antibacterial, antiviral, antifungal, sedative and analgesic effects. The gel bioactivity can be grouped according to some main action types (Grindlay & Reynolds, 1986):

— *Detoxification*. The plant determines a normal metabolism and detoxification of the organism. The action of the active components reaches the cell level and the toxins accumulated at every organism level are released resulting a very good purification which avoids the most frequent causes of getting ill.

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— *Penetration*. Due to its active components the plant is able to penetrate deeply into the human tissues. While the water shows a low penetration capacity through the skin, the Aloe Vera acts more thoroughly and its components reach thus the damaged place.

— *Stimulation of the immunity system*. The plant acts efficiently in the defending process of the organism against the external aggressions. Consequently the affected cells are recovered within few days.

— *The saponins* are also to be found in the Aloe components. They are generally triterpenoids including one or several glycoside bonds. Supardjo (2010) pointed out the saponins that show potential toxic and anti-nutritional effects in food. Battal (2002) found that an extraction at 90-95°C for 15 min. afford saponins in a 11.58-19.58% percentage. However, Narsih *et al.* (2012) which studied the effect of the extraction process performed at 80°C for 60 min on the antioxidant activity of aloin and saponin found that the extraction under these conditions results in a decreasing of the aloin and saponine contents from 4.27% to 0.987% and from 5.43% to 0.728%, respectively. Gulia *et al.* (2009) proved that the extraction of aloin and saponin at low temperatures (50-80°C) could cause the decreasing of their anti-nutritional effect from 10.6 ppm to 1.7 ppm. Moreover, Azman *et al.* (2010) have found that the extraction at 45-100°C affords a high antioxidant amount while at 120°C this amount is quite low.

— *Among the vitamins* in the plant, the following deserve mention: A, B1, B2, B6, B12, C and E. High amounts of minerals can also be found as given comparatively in Table 1 for the, *Arborescens* and *Aloe Barbadensis Miller* species (Pierce, 2000).

**Table 1**  
Amounts of Minerals in Two Aloe Species

	<i>Arborescens</i> mg/100 ml.	<i>Aloe Barbadensis Miller</i> mg/100 ml.
Calcium	103	12.76
Magnesium	69	5.86
Potassium	287	46.32
Sodium	23	24.27
Zinc	0.46	0.65
Mangenesse	5.6	1.19
Iron	0.4	0.08
Selenium	20	19
Boron	103	79
Copper	0.02	0.004
Proteins	7.3	0



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Other substances were also identified in the Aloe Vera leaf such as: sterols (cholesterinum, sitosterol, campesterol, lupeol), hormones (auxins and gibberellins), lignin, responsible for the antibiotic, analgesic antiviral and anti-inflammatory properties of the plant. Quite low amounts of Xantan gum and sorbitol have also been identified (Battal, 2002; Pierce, 2000).

## APPLICATIONS

*Medical applications.* Studies on the therapeutical applications of the plant have been performed long time ago. Thus, Sims *et al.* (1971) have performed in 1971 a series of tests to determine the positive potential of the plant on the bacteria and viruses and demonstrated that many of them were destroyed in a proportion of 80-90% in less than 72 h after an Aloe Vera gel was applied. The authors have also performed studies on the plant effect on “*Staphylococcus aureus*” (one of the most virulent pathogen germs in staphylococcus infections), “*Streptococcus viridans*” and “*Candida albicans*” (responsible for the inflammation of the mucous membrane especially in the oral and throat cavities. The obtained results afforded the following conclusions :

– The Aloe Vera gel of the 70% concentration is very efficient in curing the tissue infected with *Staphylococcus aureus* and *Streptococcus Viridans*.

– Even a concentration of 50% of the Aloe Vera gel is sufficient for a drastic diminution of *Candida albicans*.

Sims *et al.* (1971) have performed a clinical study on the effect of Aloe Vera gel administrated along with the vitamins A and E. The results thus obtained were indicative of the Aloe Vera gel effect of increasing the bioavailability of the vitamins in organism.

Other clinical studies on the Aloe Vera gel action were directed to the diabetes, cancer, some minor burns, wounds, psoriasis, acne and shingles. Following these studies the conclusion was drawn that the daily constant using of the Aloe Vera juice maintains a healthy organism due to the anthraquinones, enzymes, mucilage, vitamins and phytohormones contained (Battal, 2002).

*In cosmetic field.* It is quite advantageous to any person to know the using possibilities and benefits of the Aloe Vera plant. It is important due to its anti-inflammatory, soothing, bracing, hydrating and protection effects on the skin along with delaying the aging marks (Morrow *et al.*, 2000). Due to its properties Aloe Vera is used to prepare large number of skin care products such as (Youngken, 2000; Meadows, 1980):

- *Body and hand creams.* The Aloe Vera gel in these products improves the skin self-hydrating capacity, promotes the removal of the dead cells and assures the through absorption of the active components within the skin layers. The action mechanism

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on the skin is not entirely clear but the benefit effects of the components are well known.

The hydrating effect is caused by certain amino acids and sugars promoting indirectly the epidermis revitalization. The Aloe Vera gel in various body and hand creams is also benefit since in also acts as a emolient. The mucilage in the gel acts also as a skin protection barrier against the external factors.

- *Hair care products.* When the Aloe Vera gel is used as a main component in these products the contained active components stimulates the regeneration of the superior layer of the scalp that is intensively hydrated. Apart from this, the juice in the leaves also acts against the headache due to the anti-inflammatory effect of the tannins and saponins in the composition.
- *Soaps.* These products are quite beneficial since the gel acts against the skin drying and irritation as it happens with the glycerol containing soaps.
- *Solutions for sun protection.* A natural solution protecting the skin against the noxious sun rays can be obtained by mixing Aloe Vera gel, sesame oil, vitamin E and few drops of lavender essential oil. A calming effect is also exerted by this mixture.
- *Body wash lotions.* The products containing Aloe Vera and green lemon extracts nourish and protect the skin from the very beginning and the freshness fragrance assures the vitality along the whole day.
- *Deodorants.* As a component of deodorants the Aloe Vera gel retains the moisture so that the skin remains dry along the whole day.
- *Cosmetic masks.* Those products are proper for the mixed and fat complexions recovering the natural brightness and slowing the sebum production.

## CONCLUSIONS

The good features of the Aloe Vera confirmed by the studies in the field make this plant quite valuable as a source of nutrients for the human organism as well as a good. Ingredient for pharmaceuticals and cosmetics.



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## REFERENCES

1. Terry Laboratories, *Aloe Vera Gel for Cosmetics and Dermatology*. Quality Aloe Vera extracts & Concentrates, 17-22 (2012).
2. Adushan P., *Synthesis and Biological Activity of Aloin Derivates*. Tesis Master of Science, n the School of Chemistry University of KwaZulu, 202-211 (2008).
3. Alupului A., Calinescu I., Lavric V., *Ultrasonic vs. Microwave Extraction Intensification of Active Principles from Medicinal Plants*. University Politehnica of Bucharest, Romania, 77-79 (2007).
4. Azman M., Abdul R., Jailani S., Mashitah M.Y., Ibrahim A.B., Mohd R.M.D., *Effect of Temperature and Time to the Antioxidant Activity in Air 8 Plecranthus amboinicus Lour*. Journal American Science Terapan, 7, 9, 1195-1199 (2010).
5. Battal H., *A Research on The Production of Soapwort Extract*. Master Thesis, Ankara University Graduate School of Natural and Applied Sciences, Turkey, 161-164 (2002).
6. Bozzi A., Perrin C., Austin S., Arce Vera F., *Quality and Authenticity of Commercial Aloe Vera Gel Powders*, Food Chemistry, 103, 1, 22-30 (2006).
7. Bruneton J., *Pharmacognosy, Phytochemistry, Medicinal Plants*, Paris, Lavoisier, 562-571 (1995).
8. Cojan L., *Aloe Vera, Heath Plant*, Evenimentul, 2-3 (2012).
9. Grindlay D., Reynolds T., *The Aloe Vera Phenomenon: Phytomedicine*, 209-219 (1986).
10. Gulia A., Sharma H.K., Sarkar B.C., Upadhyay A., Shitandi A., *Changes in Physico-Chemical and Functional Properties During Convective Drying of Aloe Vera (Aloe Barbadensis) Leaves*. Food and Bioproducts Processing, 51-55 (2009).
11. Hänsel R., *Hagers Handbuch der Pharmazeutischen Praxis*. Vol. 6, Ed. Berline Springer (1994).
12. Lakshmi P.T.V., Rajalakshami P., *Identification of Phtyo-Components and its Biological Activities of Aloe Vera through the Gas-Chromatography-Spectrofotometry*. International Research Journal of Pharmacy, 871-881 (2011).
13. Lee K.Y., Weintraub S.T., *Free Radical*. Biology & Medicine, 203-212 (2016).
14. Meedows T.P., *Aloe as a Humectant in New Skin Sreparations*. Cosmetics and Toiletries, **95**, 51.56 (1980).
15. Miladi S., Damak M., *In Vitro Antioxidant Activities of Aloe Vera Leaf Skin Extracts*. Journal Société Chimique de Tunisie, 2, 542-552 (2008).
16. Narsih S., Kumalaningsih W., Susinggih W., *Identification of Aloin and Saponin and Chemical Composition of Volatile Constituents from Aloe Vera (L) Peel*. Journal of Agriculture and Food Technology, 322-331 (2012).

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17. Newton L.E., *In Defense of the Name Aloe Vera*. The Cactus and Succulent. Journal of Great Britain, 54-64 (1979).
  18. Pierce R.F., *Comparison Between the Nutritional Contents of the Aloe Gel from Conventional and Hydroponically Grown Plants*. Erde International, 5, 333-339 (2000).
  19. Sims P., Ruth M., Zimmerman E.R., *Effect of Aloevera on Herps Simplex and Herps Virus (Strain Zoster)*. Aloe Vera of American Archives, 1, 239-240 (1971).
  20. Singh B., Yadav R., Singh H., Singh G., Punia A., *Studies on Effect of PCR-RAPD Conditions for Molecular Analysis in Asparagus (Satawari) and Aloe Vera Medicinal Plants*. Australian Journal of Basic and Applied Science, 4, 6570-6574 (2010).
  21. Superdjo A., *The Effect of Saponin to Livestock and Human Life*. Animal Husbandary Faculty. Journal of the Jambi University, 76-81 (2010).
  22. Tucker A.O., Duke J.A., Foster S., *Botanical Nomenclature of Medicinal Plants*. Eds, Herbs, Spices and Medicinal Plants, Vol. 4, Phoenix, AR, Oryx Press, 169-242 (1989).
  23. Tvet P.T., *Identification of Phtyo-Components of Aloe Vera Through the Gas-Chromatography-Spectrofotometry*. Food Chemistry, 103, 22-30 (1903).
  24. Wang G.H., Su P., Zhang F., Hou Xiang Y., Yang Y., Guo Z.K., *Comparison of Microwave-Assisted Extraction of Aloe-Emodin in Aloe with Soxhlet Extraction and Ultrasound-Assisted Extraction*. Science China Chemistry, 555-564 (2011).
  25. Youngken H.W., *Textbook of Pharmacognosy and Cosmetics*. Philadelphia, Blakiston, 872-873 (2000).
  26. Zont F., Bogoni P., Masotti P., Micali G., *High-Performance Liquid Chromatographi Profiles of Aloe Vera Constituents and Determination of Aloin in Beverages, with Reference to the EEC Regulation for Flavouring Substances*. Biotherm, 23-30 (2000).

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Ack. : BULETINUL INSTITUTULUI POLITEHNIC DIN IAȘI Publicat de Universitatea Tehnică "Gheorghe Asachi" din Iași  
Tomul LIX (LXIII) Fasc. 4, 2013.

## 2015 New Year's Greeting

### An Age of Competitive Collaboration

At the beginning of 2015, it is my pleasure to express to you my best wishes for the New Year and pray that all of you will continue your good work in good health.

With the rapid globalization we have seen our surroundings politically, economically, and culturally standardized all over the world mostly under duress. Now is the time when our ability and capability will be put to the test not only as a scientist but also a human being. Our field of science is no exception. Ironically, it is only through free and fair competition with others that we can achieve our goal avoiding unnecessary threat or fear. This is the very reason, I believe, why we need to collaborate with each other in order to survive or coexist in this too complicated a world full of diversities and difficulties. The more intensified competition becomes, the closer international understanding and collaboration is required. In another word, to acquire mutual mature development both strengthening strong points and at the same time making up for weak points are essential elements.



**Mitsuo Miyazawa**  
President of JOCS

In such a world what is needed are :

- 1) clear understanding of globalization with world-wide perspectives.
- 2) originality (creative thinking with challenging suggestion and practice).
- 3) ability (capability to face and solve problems).
- 4) Charity (integrated personality with keen awareness of our role as a scientist.

Combining together these elements I mentioned here, I'd like to call it "Competitive Collaboration".

These years another goal of our society has been to build up and deepen good relationship with Asian scientists. To develop our JOCS in Asia into more flourishing and trustworthy one, let's a try to get deeper understanding through more frequent communication and with "give and take" spirit. Let's keep on moving taking a step forward.

Next, let me allow to introduce two episodes in my life, (one is from an article of a local paper, the other is from my own experience).

- 1) "a place of dream and hope".

The other day I happened to read the following article in a local paper, in which an architect was asked to design a school for the disabled students. His first intention was

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to design a functional school building with such facilities as gentle declining straight slopes and standard elevators. His comprehension of user-friendly design was 'simplicity and functionality'. But what he got from students as requests was quite different from what he had imagined.

"My dream elevator is like this: I can enjoy the landscape while moving up and down in it."

"From time to time I feel like going up and down all by myself."

"It'll be fun to move on a spiral slope where we can watch what is going on in every classroom, and what's more, we can enjoy free chat with friends."

The students proposed their plans full of dreams one after another. The architect learned: to design pursuing functionality and simplicity is one thing and to answer their needs is quite another. He had lost playful spirit or sense of flexibility in his work. After all he managed to change his first plan to a spiral slopes and glass elevators. Furthermore, after 3.11 Earth Quake in 2011, his building played an important role as a safe haven for the local people.

2) "See! What I mean?"

In 1985 I went to the U.S. to study for two and half years as a Ph.D researcher at MSU (Michigan State University). One day after I finished my daily routine together with my foreign colleagues. I went home as usual. That night my teacher received a call from a Radio-Isotope inspector notifying him of complaints about the contamination of our room. The next morning my teacher gave me a minute explanation of what I had done. Of course I knew I was to blame, so I was determined to handle it all by myself. To my astonishment, he was the first to restore the contaminated place to former condition, saying nothing but "Mitsuo! See, what I mean ? After that he was always with us and trained us with friendly and fatherly attitude. After a long time, every time I celebrate the New Year, I find myself recalling the scene and his gentle smile and voice of encouragement. I made up my mind to hand down what I learned from him to young scientists of mine.

Inclosing, let me add a few words. At present our society is preparing several awards. Both popularized and professionalized science have a tendency to join hands with industrial worlds or businesses in order to win public supports. But please keep in your mind our first and foremost principle, that is, we should start our study or research from the basics, namely, "elucidation of nature" and end up with a contribution to human welfare. I do hope you consider again the meaning of these words and stick to our original purpose.

*(Prof. of Kinki (Kindai) University)*

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## PARLIAMENT NEWS

Lok Sabha Unstarred Question No. 3628 - Answered on 05.08.2014

### Subsidy for Cultivation of Pulses and Oilseeds

Shri Innocent

Will the Minister of Agriculture be pleased to state :

- (a) whether the Government proposes to provide subsidy to promote the cultivation of pulses and oilseeds in the country, and*
- (b) if so, the details thereof and if not, the reasons therefor ?*

#### **Answer**

Minister of State in the Ministry of Agriculture and Food Processing Industries  
(Dr. Sanjeev Kumar Balyan)

(a) Government is providing assistance to promote cultivation of pulses and oilseeds in the country under crop development schemes like National Food Security Mission (NFSM), National Mission on Oilseeds and Oil Palm (NMOOP) and Rashtriya Krishi Vikas Yojana (RKVY).

(b) Under these programmes, assistance is provided for activities like demonstration of improved technologies, distribution of improved seeds; need based plant protection and soil amendments including bio-fertilizers & bio-pesticides, improved farm implements; resource conservation techniques, efficient water application tools; farmers' trainings; etc for enhancing production and productivity of pulses and oilseeds.

Lok Sabha Unstarred Question No. 3618 - Answered on 05.08.2014

### Production of Oilseeds

Shri Pashupati Nath Singh/  
Shri Harishchandra Chavan

Will the Minister of Agriculture be pleased to state :

- (a) whether the Government is aware of decline in the production of oilseeds due to plant diseases and insects in the country,*
- (b) if so, the details thereof; and*
- (c) whether the current production of oilseeds is inadequate to meet the demand of growing population in the country and if so, the details thereof and the steps taken by the Government to address the said issues ?*

#### **Answer**

Minister of State in the Ministry of Agriculture and Food Processing Industries

(a) & (b) : Production of oilseeds fluctuates depending upon rainfall, as these crops are largely grown under rainfed conditions. Oilseeds production has increased from 29.80 million tonnes in 2011-12 to 32.41 million tonnes during 2013-14 (3rd advance estimates).

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(c) : The production of oilseeds in 2012-13 was 30.94 million tonnes as against the requirement of 66.55 million tonnes and therefore, has not been able to meet the demand of the growing population in the country.

In order to increase the production and productivity of oilseeds, Government of India has launched the National Mission on Oilseeds and Oil Palm in 2014-15 in the country.

## **Government Notifications**

### **Determination of Rate of Exchange for Determining Value of Taxable Service**

Government of India  
Ministry of Finance  
(Department of Revenue)

Notification No. 19/2014-Service Tax

New Delhi, the 25th August, 2014

G.S.R..... (E). – In exercise of the powers conferred by sub-section (1) read with sub-section (2) of section 94 of the Finance Act, 1994 (32 of 1994), the Central Government hereby makes the following rules further to amend the Service Tax Rules, 1994, namely:-

1. (1) These rules may be called the **Service Tax (Second Amendment) Rules, 2014.**  
(2) They shall come into force on the **1st day of October, 2014.**

2. In the Service Tax Rules, 1994, after rule 10, the following rules shall be inserted, namely :-

11. Determination of rate of exchange – The rate of exchange for determination of value of taxable service shall be the applicable rate of exchange as per the generally accepted accounting principles on the date when point of taxation arise in terms of the Point of Taxation Rules, 2011.

12. Power to issue supplementary instructions – The Board or the Chief Commissioners of Central Excise may issue instructions for any incidental or supplemental matters for the implementation of the Provisions of Act.

Sd/-

(Akshay Joshi)

Under Secretary to the Government of India

[F. No. 334 / 15 / 2014-TRU]

**Note :-** The principal rules were published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (i) vide notification No. 2/94-SERVICE TAX, dated the 28th June, 1994 vide number G.S.R. 546 (E), dated the 28th June, 1994 and last amended vide notification No. 9/2014-Service Tax, dated the 11th July, 2014 vide number G.S.R. 478(E), dated the 11th July, 2014.



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## Defination of “Intermediary” in Service Tax

Government of India  
Ministry of Finance  
(Department of Revenue)

Notification No. 14/2014-Service Tax

New Delhi, the 11th July, 2014

G.S.R..... (E). – In exercise of the powers conferred by sub-section (1) of section 66C and clause (hhh) of sub-section (2) of section 94 of the Finance Act, 1994 (32 of 1994), the Central Government hereby makes the following rules to amend the Place of Provision of Services Rules, 2012, namely:–

1. (1) These rules may be called the Place of Provision of Services (Amendment) Rules, 2014.  
(2) They shall come into force on the 1st day of October, 2014.
2. In the Place of Provision of Services Rules, 2012 : –
  - (a) in rule 2 for clause (f), the following clause shall be substituted, namely :–
    - (f) “intermediary” means a broker, an agent or any other person, by whatever name called, who arranges or facilitates a provision of a service (hereinafter called the “main” service) or a supply of goods, between two or more persons, but does not include a person who provides the main service or supplies the goods on his account;“;
  - (b) in rule 4, in clause (a), for the second provision, the following provision shall be substituted, namely :–

“Provided further that this clause shall not apply in the case of a service provided in respect of goods that are temporarily imported into India for repair and are exported after the repairs without being put to any use in the taxable territory, other than that which is required for such repair;“;
  - (c) in rule 9, for clause (d) the following clause shall be substituted, namely :–
    - (d) Service consisting of hiring of all means of transport other then, –
      - (i) aircrafts, and
      - (ii) vessels except yachts,  
upto a period of one month.”

[F. No. 334 / 15 / 2014-TRU]

(Akshay Joshi)

Under Secretary to the Government of India

**Note :-** The principal notification was published in the Gazette of India, Extraordinary, by notification No. 28/2012 – Service Tax, dated the 20th June, 2012 vide number G.S.R. 470 (E), dated the 20th June, 2012.

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## **“Provider of Taxable Service” Inserted in CENVAT Credit Rules**

Government of India  
Ministry of Finance  
(Department of Revenue)

Notification No. 25/2014-Central Excise (N.T.)

New Delhi, the 25th August, 2014

G.S.R..... (E). – In exercise of the powers conferred by section 37 of the Central Excise Act, 1944 (1 of 1944) and section 94 of the Finance Act, 1994 (32 of 1994), the Central Government hereby makes the following rules further to amend the CENVAT Credit Rules, 2004, namely :–

1. (1) These rules may be called the CENVAT Credit (Seventh Amendment) Rules, 2014.  
(2) They shall come into force on the date of their publication in the Official Gazette.
2. In the CENVAT Credit Rules, 2004, in rule 12AAA, after the words “first stage and second stage dealer”, the words “provider of taxable service” shall be inserted.

Sd/-  
(Akshay Joshi)  
Under Secretary to the Government of India

[F. No. 334 / 15 / 2014-TRU]

**Note :-** The principal rules were published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (i), dated the 10th September, 2004 vide notification No. 23/2004-Central Excise (N.T.), dated the 10th September, 2004 vide number G.S.R. 600 (E), dated the 10th September, 2004 and last amended vide notification No. 21/2014 - Central Excise (N.T.), dated 11th July, 2014 published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (i), vide number G.S.R. 456 (E), dated the 11th July, 2014.



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## PARLIAMENT NEWS

Rajya Sabha Unstarred Question No. 641 - Answered on 28th November 2014

### DECLINE IN SOWING OF OIL SEEDS

Shri Rajkumar Dhoot

Will the Minister of Agriculture be pleased to state :

- (a) *whether it is a fact that sowing of oil seeds has considerably decreased in Maharashtra and other parts of the country during the current crop season;*
- (b) *if so, the details thereof;*
- (c) *whether this would adversely affect the production of oilseeds in the country;*
- (d) *if so, the details thereof; and*
- (e) *the action Government has taken in the matter ?*

#### **Answer**

Minister of State in the Ministry of Agriculture  
Shri Mohanbhai Kundaria

(a) & (b) : In Maharashtra, the area coverage under oilseeds during kharif 2014 was higher than normal kharif area under oil seeds in the State. However, at all India level, kharif coverage of oilseeds was lower than normal kharif area due to late onset of monsoon and drought like situation in various States. Rabi sowing of oil seeds is in progress. The normal and current year area coverage of oil seeds in respect of Maharashtra and all India are given below :—

Year	Maharashtra		All India	
	Kharif	Rabi	Kharif	Rabi
Normal	35.58	3.3	185.8	83.2
2014-15*	39.14	0.4	176.8	54.0

\* Area coverage, as on 13/11/2014.

(c) & (d) : As per the 1st advance estimates, all India oil seeds production during kharif 2014 is estimated to be 196.64 lakh tonnes against target of 218.30 lakh tonnes. The target of production of oil seeds in rabi season is 111.70 lakh tonnes. Slowing in rabi season is in progress.

The oil seeds production during kharif 2014 in Maharashtra is estimated to be 43.70 lakh tonnes against target of 49.82 lakh tonnes. The target of rabi oil seeds production is 2.68 lakh tonnes.

(e) : To increase production and productivity of oil seeds, National Mission on oil seeds and Oil Palm (NMOOP) is being implemented in the country from 2014-15.

**IMPORT OF OIL SEEDS**

Shri Arka Keshari Deo

Will the Minister of Commerce & Industry be pleased to state :

- (a) whether the Government has any plan / proposal to import oil seeds to meet country's demand;*
- (b) if so, the details thereof;*
- (c) the likely impact of such import on domestic market in the country;*

**Answer**

The Minister of State in the Ministry of Commerce and Industry (Independent Charge)  
Smt. Nirmala Sitharaman

(a) Decisions on importing agricultural products including oilseeds are taken by the Government keeping in view several factors viz. domestic demand and production, domestic and international prices, likely effect on the domestic producers and consumers etc. Currently, Department of Commerce has no plan of importing oilseeds. However, import Policy for oilseeds (other than that of seed quality and copra) classified under Chapter 12 of ITC HS Code list is 'free' subject to conditions prescribed in the Exim Policy and payment of custom tariff at applicable rates.

(b) & (c) Does not arise in view of (a) above.

**PRODUCTION OF OILSEEDS AND PULSES**

Shri Arka Keshari Deo;

Shri M. Raja Mohan Reddy;

Shri Pralhad Joshi;

Shri Nalin Kumar Kateel;

Shri Chand Nath

Will the Minister of Agriculture be pleased to state :

- (a) the details of the production and the area under cultivation of oilseeds and pulses in the country during each of the last three years and the current year, Statewise;*
- (b) whether the Government has assessed the production and consumption of oilseeds and pulses during the above period and if so, the details thereof, crop-wise;*

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- (c) whether there is a gap between production and consumption of these crops;
- (d) if so, the details thereof; and
- (e) the steps taken by the Government to encourage the farmers and incentives / assistance / subsidy given to them as well as agencies concerned to produce more oilseeds and pulses to meet the demand and bridge the gap between the production and consumption of these crops in the country ?

**Answer**

Minister of State in the Ministry of Agriculture  
Dr. Sanjeev Kumar Balyan

(a) State-wise details of production and area under cultivation of oilseeds and pulses in the country during the last three years and current year, i.e. 2011-12 to 2014-15 are given in the Annexure.

(b) to (d) : Household consumption of goods and services in the country is assessed through Household Consumer Expenditure Surveys conducted by National Sample Survey Office (NSSO) of Ministry of Statistics and Programme Implementation. Based upon per capita monthly quantity of consumption as per Household Consumer Expenditure Survey: 2011-12 (latest available), the details of total consumption vis-a-vis production of oilseeds and pulses in the country during 2011-12 to 2013-14 are as under :

**(Million Tonnes)**

<b>Crop</b>	<b>Year</b>	<b>Production</b>	<b>Consumption</b>
Oilseeds	2011-12	29.80	37.72
	2012-13	30.94	38.23
	2013-14	32.88	38.74
Pulses	2011-12	17.09	11.79
	2012-13	18.34	11.95
	2013-14	19.27	12.10

- Note
1. Annual production of oilseeds & pulses for 2014-15 are not available.
  2. Production figures for 2013-14 are as per 4th Advance Estimates.
  3. Consumption figures only relate to consumption by household and do not include indirect consumption.
  4. Consumption of oilseeds also includes consumption of edible oils based upon the recovery rate of 28% from oilseeds to edible oils.
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(e) To encourage farmers to produce more oilseeds and pulses to bridge the gap between their production and consumption, Government of India has been implementing various Crop Development Schemes such as National Food Security Mission (NFSM) - Pulses, National Mission on Oilseeds and Oil Palm (NMOOP), Rashtriya Krishi Vikas Yojana etc. Under the above schemes, assistance is provided for purchase of breeder seed, production and distribution of certified seed, planting material, establishment of seed gardens of oil palm, distribution of production inputs, transfer of technology through block demonstrations, integrated pest management, supply of plant protection equipments / chemicals, farmers training etc.

In order to increase production and productivity of oilseeds and pulses, the Indian Council of Agricultural Research (ICAR) is conducting research programmes on different oilseeds and pulses crops being undertaken by five commodity based research institutes and 10 All India Coordinated Research Projects on specific oilseeds and pulses for developing location-specific varieties / hybrids and suitable technologies. During the last five years (2009-2013), ICAR has released 97 varieties/hybrids of different oilseeds and 89 varieties of different pulses that have fair degree of tolerance to biotic and abiotic stresses.

## Annexure

Annexure referred to in reply to parts (a) and (b) of Lok Sabha Unstarred Question No.354 due for answer on 25.11.2014.

**State-wise Area of Oilseeds during 2011-12 to 2014-15**

('000 Hectares)

State / UT	Oilseeds			
	2011-12	2012-13	2013-14*	2014-15\$
Andhra Pradesh	1945.0	1945.0	1966.0	848.0
Arunachal Pradesh	32.5	32.3	#	#
Assam	268.3	306.2	284.0	21.0
Bihar	133.4	128.0	129.4	5.9
Chhattisgarh	308.3	297.5	289.7	211.4
Goa	3.2	3.1	#	#
Gujarat	3131.0	2452.0	3078.0	2240.0
Haryana	553.0	580.2	551.2	20.0
Himachal Pradesh	14.9	13.5	16.2	3.7
Jammu & Kashmir	64.6	64.8	65.2	4.9
Jharkhand	228.9	250.6	269.7	34.9
Karnataka	1416.0	1422.0	1507.0	857.0
Kerala	1.9	1.0	0.9	0.4
Madhya Pradesh	7201.6	7534.4	7828.0	6212.4
Maharashtra	3667.0	3806.0	4454.0	3914.0
Manipur	35.9	44.1	#	#
Meghalaya	9.9	10.0	#	#
Mizoram	2.5	2.1	#	#
Nagaland	64.0	64.5	#	#
Odisha	250.7	243.3	221.6	140.6
Punjab	50.0	51.4	49.4	19.0
Rajasthan	4622.7	4912.2	5279.8	1802.9
Sikkim	9.3	8.2	#	#
Tamil Nadu	449.2	388.5	421.8	220.9
Telengana	-	-	-	252.0
Tripura	4.7	4.8	#	#
Uttar Pradesh	1129.0	1147.0	1107.0	472.0
Uttarakhand	30.0	32.2	32.0	18.0
West Bengal	676.1	732.1	786.7	225.9
D&N Haveli	0.2	0.1	#	#
Delhi	4.0	6.8	#	#
A&N Islands	NG	NG	NG	NG
Pondicherry	0.4	0.4	#	#
Others	NA	NA	187.7	59.9
<b>All India</b>	<b>26308.2</b>	<b>26484.4</b>	<b>28525.3</b>	<b>17674.6</b>

\* 4th advance estimates; \$ 1stb advance estimates (Kharif only); # included in others; N G Not Grain; NA not Applicable.

## Annexure

Annexure referred to in reply to parts (a) and (b) of Lok Sabha Unstarred Question No.354 due for answer on 25.11.2014.

**State-wise Production of Oilseeds**

('000 Hectares)

State / UT	Oilseeds			
	2011-12	2012-13	2013-14*	2014-15\$
Andhra Pradesh	1264.7	1651.1	1836.0	422.0
Arunachal Pradesh	33.0	30.8	#	#
Assam	149.5	186.8	162.2	13.0
Bihar	139.5	143.3	144.1	6.4
Chhattisgarh	169.5	215.0	185.4	143.3
Goa	8.0	7.5	#	#
Gujarat	5035.0	2705.0	6837.9	4229.3
Haryana	771.0	993.1	898.5	24.5
Himachal Pradesh	8.6	6.9	9.2	2.0
Jammu & Kashmir	53.4	51.1	51.4	2.1
Jharkhand	155.5	197.2	192.9	34.4
Karnataka	942.0	919.6	1284.9	568.0
Kerala	2.4	1.1	1.0	0.5
Madhya Pradesh	7727.8	9276.0	6655.0	6560.7
Maharashtra	4485.0	5086.8	5241.0	4369.4
Manipur	28.3	32.1	#	#
Meghalaya	7.6	6.9	#	#
Mizoram	2.4	2.2	#	#
Nagaland	66.8	67.5	#	#
Odisha	165.8	170.3	161.2	68.7
Punjab	68.0	69.4	67.8	27.0
Rajasthan	5744.5	6364.6	6069.8	2045.5
Sikkim	7.8	7.1	#	#
Tamil Nadu	1113.7	816.9	1004.9	468.7
Telengana	-	-	-	245.8
Tripura	3.5	2.4	#	#
Uttar Pradesh	935.0	1030.5	932.1	139.0
Uttarakhand	32.5	39.7	36.0	27.0
West Bengal	672.4	850.7	932.9	217.2
D&N Haveli	0.1	0.1	#	#
Delhi	4.9	8.6	#	#
A&N Islands	NG	NG	NG	NG
Pondicherry	0.5	0.8	#	#
Others	NA	NA	172.8	49.8
<b>All India</b>	<b>29798.7</b>	<b>30941.1</b>	<b>32876.8</b>	<b>19664.2</b>

\* 4th advance estimates; \$ 1stb advance estimates (Kharif only); # included in others; N G Not Grain; NA not Applicable.



### **CULTIVATION OF PALM**

Dr. Nepal Singh

Will the Minister of Agriculture be pleased to state:

*(a) whether the Government has formulated any scheme to promote the cultivation of Palm across the country.*

*(b) if so, the details thereof, State-wise; and*

*(c) the success achieved in increasing the production of Palm therefrom ?*

#### **Answer**

The Minister of State in the Ministry of Agriculture

Dr. Sanjeev Kumar Balyan

(a) to (c) Yes, Madam. In order to promote cultivation of oil palm in the country, Government of India implemented Oil Palm Development Programme (OPDP) from 1991-92 to 2003-04, Integrated Scheme for Oilseeds, Pulses, Oil Palm and Maize (ISOPOM) from 2004-05 to 2013-14 and Oil Palm Area Expansion (OPAE) from 2011-12 to 2013-14. In XIIth Five Year Plan, National Mission on Oilseeds and Oil Palm (NMOOP) is being implemented from 2014-15.

Under Mini Mission-II (Oil Palm) of NMOOP, assistance is provided for purchase of planting material, maintenance for gestation period, installation of drip-irrigation system, diesel/ electric pump-sets, bore-well / water harvesting structure / ponds, inputs for intercropping in oil palm fields, construction of vermi-compost units, training, demonstration, establishment of new seed garden, purchasing of machinery & tools and setting up of oil palm processing units in NE/hilly States/LW areas. Directorate of Oil Palm Research (DOPR), Pedavegi, ICAR is providing technical guidance to the implementing States and also supplying the planting material of oil palm.

Area under oil palm cultivation has increased from 8,585 hectare in 1991-92 to 2,50,763 hectare in 2013-14. Similarly, the production of fresh fruit bunches (FFBs) and crude palm oil (CPO) was 21,233 MT and 1,134 MT respectively during the year 1992-93, which has now increased to 9,95,211 MT of FFBs and 1,71,354 MT of CPO during the year 2013-14. During the year 2014-15, Government of India has fixed a target to cover 34,750 hectares are under oil palm in 14 States. The State-wise details are given in Annexure.

**Annexure refer to Question No. 270 for answer on 25.11.2014**

<b>Sl. No.</b>	<b>State</b>	<b>Existing Area under Oil Palm</b>	<b>New Oil Palm area expansion target (in ha.) during 2014-15</b>	<b>Funds outlay for 2014-15 (Rs. in crores)</b>
1.	Arunachal Pradesh	1,30,531	14000	44.80
2.	Telangana	14,796	2000	7.45
3.	Karnataka	31,549	3100	10.13
4.	Tamil Nadu	22,854	1800	3.50
5.	Gujarat	4,196	450	1.29
6.	Odisha	16,225	3000	18.42
7.	Kerala	5,740	500	0.92
8.	Chhattisgarh	412	250	0.95
9.	Mizoram	19,971	4000	11.88
10.	Assam	10	1000	3.60
11.	Nagaland	0	1500	0.90
12.	Arunachal Pradesh	0	1600	2.44
13.	Meghalaya	0	1000	0.51
14.	West Bengal	0	550	0.52
15.	Other States	4,479	0.00	0.00
	<b>Total</b>	<b>2,50,763</b>	<b>34750</b>	<b>107.31</b>

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***Prof. D. K. Bhattacharyya***

M. Sc., (Tech), Ph. D  
Emeritus Fellow (AICTE)  
Ex-Ghosh Professor of Applied Chemistry

DEPARTMENT OF CHEMICAL TECHNOLOGY  
University Colleges of Science & Technology  
University of Calcutta  
92, Acharya Prafulla Chandra Road  
Kolkata - 700 009  
Phone : 2350 8386/6396/6387/1857/1014/9937  
2352 0051/0052  
Gram : SCIENCE Fax : 91-033-351 9755  
E-mail : dkb\_oiltech@yahoo.com

## **A REVIEW**

*The book entitled “A treatise on Analysis of Food, Fats and Oils” is an example of unique competence and contribution of the authors, S. K. Roy, N. K. Pramanik and A. R. Sen.*

*The book is the first of its kind in India. It covers the traditional and modern analytical methods for the characterization and quality of fats, oils as well as other food items.*

*The authors are well reputed and qualified and they have applied their collective wisdom and expertise in including and presenting more appropriately and meticulously the analytical methods.*

*The book can also be viewed as a rarer type as it deals with the statutory and industrial aspects of fats, oils and their products, and pollution control in vegetable oil industry.*

*In fact these aspects are of extreme use and importance to those concerned with these issues.*

*The book is already well received by the readers and users in the academic and industrial circles throughout India because of the highly relevant and beneficial methodologies and basic-cum technological information. The book will be recognised in due course of time as one of the top quality analytical books in the area of food, fats and oils.*

***Prof. D. K. Bhattacharyya***

**21-6-2003**

Regarding availability/price enquiries may be made to :  
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es.k.ray@gmail.com

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## BOOK REVIEW

A book entitled “Perfumery Materials, Production and Applications” has been authored by an very eminent Professor (Dr) D. K. Bhattacharyya, Emeritus Fellow (AICTE), Adjunct Professor Bengal Engineering and Science University, former President, O.T.A.I and a Scientist of National and International repute.

The book speaks for itself about his mastery and competence in the discipline of “Perfumery Materials”.

“The book demonstrates the scopes of certain specific reactions and raw materials in producing new synthetics. The enormous scopes of biotechnology involving bio-conversion processes’, with isolated enzymes and by fermentation biotechnology involving selective microorganisms has been indicated in making synthetics. The applications of natural aromatic oils in aromatherapy, food, cosmetics/toiletries, imitation perfumery and allied sector have been included.

Standardisation and evaluation of natural aromatic (essential oils and incidence of their adulteration have been elaborated in order to ascertain their quality and authenticity for sustaining the business in the industry” says Prof (Dr) R.N. Mukherjee, Former, Professor and Head, Deptt of Chemical Engg, University of Jadavpur. The book will fulfill a long felt want in the discipline of Essential Oils and will cater to the various categories of Scholars, Scientists and Technologists. The book has already been well appreciated in India and abroad, though published by the Stadium Press L.L.C., USA.

Those interested to procure a copy of this Valued book on Essential Oils may contact Professor D. K. Bhattacharyya at Phone No (033) 2461 9662.

(S. K. Roy)  
Editor

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