

OIL TECHNOLOGISTS' ASSOCIATION OF INDIA

MARCH 2020 - AUGUST 2021

EASTERN REGION



FOR LIMITED CIRCULATION



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From the Editorial Perspective



It is after a long gap we are publishing our News Letter. The reason behind as we all know is Pandemic Covid 19. which has brought Life to a stand still.

Enormous damage has been done to Human lives and may people have lost their near and dear ones.

We have lost a very valuable OTAI MEMBER HGS Mr .Dhirendra Tewari (our Heartfelt Condolences to the bereaved family members**OmShanti!!)

Around the world scientific community with their relentless research have finally developed some "Vaccine" to counter this pandemic.

OTAI.E.R Calcutta even in these circumstances could hold a couple of meetings in Physical mode and Dr Santi Nath Ghosh Memorial Research AwardLecture by Dr Debjyoti in virtual mode.

Dr Debjyoti Paul narrated a very interesting episode about his Guide.

Dr Santi Nath Ghosh asked him "What are Lipids"? And Dr Debjyoti Paul could not reply.

Dr Ghosh his guide requested him to through the "Basics".

The simple interaction between A GUIDE & SCHOLAR PROVIDES

How much value a Real guide gives to basics...that Had Been Dr Santinath Our Beloved Colleague..

Coming back to Covid 19..there are many suggestions & remedial measures in addition to Vaccine.. The best suggestion probably is Prevention is better than Cure & for that "IMMUNITY" comes in the forefront.

In this connection it may be stated that there are some reports that individuals having higher levels of Omega-3 in the blood may not come under the higher Risk of Covid-19.

Omega-3 (EPA;DHA) Comes under GRAS and consumption of Omega3 from naturally occurring resources or supplements may be a welcome move as there is imbalance in the ratio of w3:w6 in majority of individual daily fatty acid consumption.**

****IT IS NOT A PRESCRIPTION OF A QUALIFIED DOCTOR****

MY COLLEAGUES ARE REQUESTED TO FEEL FREE TO TAKE A DECISION OF THEIR OWN...OR CONSULT A PHYSICIAN **

NUTRITION IS OF PARAMOUNT IMPORTANCE FOR GOOD HEALTH. and what is NUTRITION?
NUTRITION is an input to and Foundation for Good Health

With Prayers and Wishes for Good Health for you & Family members

S. K. Roy
Editor

*COVID-19 Protocol

COVID - 19 PROTOCOLS

HOME QUARANTINE ADVICE FOR COVID PATIENT (For Asymptomatic / with mild symptoms)



- Covid Symptoms: Fever/ Myalgia / Headache / Cold / Cough / Dry throat / Rarely Loose motion
- Do Basic Investigation : CBC / ESR / CRP / RBS / SGOT / SGPT / RFT / Covid RT PCR / X-Ray Chest
- Get your Family physician opinion
- Your Doctor will plan your Home Isolation & Guide you.

1. Medicines (As per Doctor's Advice)

S.No	Name of the medicine	Type of medicine	Dose	Duration	Instruction
1	Tab. Azithral 500 mg	Antibiotic	1 – 0 – 0	5 days	Morning (AF)
2	Cap. Antiflu 75 mg	Antiviral	1 – 0 – 1	5 days	Morning & Evening
3	Tab. Pan 40 mg	For Acidity	1 – 0 – 1	5 days	Morning & Evening (BF)
4	Tab. Montek LC	Anti cold	0 – 0 – 1	5 days	Night (AF)
5	Tab. Zincovit	Multivitamin	1 – 0 – 0	10 days	Morning (AF)
6	Tab. Celin 500 mg	Vitamin C	1 – 0 – 1	10 days	Morning & Evening (AF)
7	Cap. Lumia 60 K	Vitamin D	1 stat	1 day only	Only One Dose
8	Tab. Dolo 650 mg	Antipyretic	1 – 1 – 1	SOS	For Pain & Headache
9	Betadine Gargle / Hot Water Gargle	Antiseptic Gargle	3 times in a day	-	-
10	Extra Medication	Steroids / Anticoagulant / Antidiabetic	If needed (Based on your Lab Test)	-	-
11	Patient with co.morbidity	Continue your Regular Medicines	-	-	-

2. Steam Inhalation - 3 Times in a day

It is considered to be useful in damaging the capsid of the SARS-CoV-2 envelope and prevent worsening of infection and spread

3. Pulse oxymeter monitoring -

- Normal oxygen saturation of blood in healthy individual is 95% to 100%.
- 5 minutes Walk Test: Check your SpO₂ level after 5 minutes walk (>95% is normal)
- Check 3 Times in a day (If having Shortness of breath → Immediately **Consult** your Doctor)

4. Temperature monitoring - (Use digital Thermometer)

5. Food

- Take rich Protein Diet and Fruits / Avoid cold items & Aerated Cool drinks
- Drink Turmeric Milk in night
- Eat Walnut and Anjeer (அத்திப்பழம்) and 2 Eggs
- Eat fresh Fruits and Vegetables
- Drink enough Water everyday
- Eat moderate amount of Fat and Oil
- Eat less Salt and Sugar

- Avoid listening and reading more news about COVID-19 / Be Positive and Be Bold
- Do Breathing Exercise (**Spirometry**) - 3 times a day
- Average recovery time is 10 to 14 days. Get another RT-PCR test after 2 weeks (If necessary-after consulting your doctor)
- No need to do CT scan of Lungs-if patient is fine / or Symptom free (As per Doctor's Advice)
- Patient having Ct values (refer To RT PCR report) between 24 to 35 is less infectious. Ct value - 15 to 23 more infectious.
- Take complete Rest and Read good Books and Articles / Listen good Music
- Stop Smoking / Stop Drinking Alcohol
- Use separate Bed room & Rest room
- Wear mask while moving your family members
- Get your family members / close contact – tested
- For Pediatric patient – Consult your Pediatrician

Wish you a speedy Recovery. God Bless You.

Any Queries, Call : 7639177700 / 8012277700(WhatsApp Number)(24x7 service) and clear your doubts.

STAY HOME / STAY ALERT / STAY SAFE / CONTROL VIRUS / WIN COVID

About ourselves

1. Dr Sibdas Bandyopadhyaya has been elected as President (East Zone) Calcutta for the term 2020-22.

2. A.G.M was held in physical mode.
Attendance however was thin due to Pandemic.

3. Dr Santinath Ghosh memorial Research Award lecture
was delivered in virtual mode by Dr Debjyoti Paul Asst Prof Techo India University on the 1st August 2021.

The Topic of the lecture was.... "A novel nano formulation of alpha-eleostearic acid restores molecular pathogenesis of hypersensitivity" in virtual mode.

It was presided over by Dr Sibdas Bandopadhyaya, President E.Z.

Among E.Z. members present were

Prof. D. K. Bhattacharyya, Dr. Pubali Ghosh Dhar, Dr. Mohua Ghosh.

2. S. K. Roy narrated the details about the inception of the Award and its implementation.

3. Mr. B. P. Manchanda gave the vote of thanks

It was well attended.

****Dr A.S.Khanna; A Lifetime Achievement Awardee and one of the founder members of the East Zone Calcutta..has left for heavenly abode on the 18th June 2021.****

Unsaturated aldehydes induce CCK secretion via TRPA1 in STC-1 cells

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Scope: Cholecystokinin (CCK) producing cells sense luminal contents to regulate the exocrine pancreas, gastric motility, and appetite. Although long-chain fatty acids (FAs, \geq C12) are well known to stimulate CCK secretion, the CCK-releasing activities of other aliphatic compounds, such as aldehydes (Alds) or alcohols (Alcs), have not been studied.

Methods and results: We tested the CCK-releasing activities of various aliphatic compounds with various carbon chain lengths (C3-C13) and degrees of unsaturation in the enteroendocrine cell line STC-1. CCK released from the cell was measured using an ELISA, and intracellular calcium concentration was measured using Fura-2. Mono- and di-unsaturated Alds at 100 μ M, but not saturated Alds, induced CCK secretion in STC-1 cells. Alcs and FAs failed to induce CCK secretion, regardless of carbon chain length or degree of unsaturation. Unsaturated Alds increased intracellular calcium concentration, but saturated Alds, Alcs, and FAs did not. Intracellular calcium mobilization and CCK secretion induced by unsaturated Alds was abolished in the absence of extracellular calcium. In addition, the inhibition of the transient receptor potential ankyrin 1 (TRPA1) channel suppressed unsaturated Ald-induced CCK secretion and intracellular calcium mobilization.

Conclusion: Unsaturated Alds are potent aliphatic stimulants for CCK secretion through the activation of TRPA1.

Keywords:

Aldehyde / CCK / TRPA1



Additional supporting information may be found in the online version of this article at the publisher's web-site

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1 Introduction

Gut hormones mediate nutrient and non-nutrient signals from the gut to target tissues that regulate gastrointestinal functions, including secretion, motility, digestion, and absorption. One of the gut hormones, cholecystokinin (CCK),

is produced in enteroendocrine cells dubbed "I cells" [1]. CCK-producing I cells are mainly localized in the proximal gut and respond to luminal contents via various sensors [2].

It has been recently demonstrated that various receptors are expressed in I cells and mediate CCK secretion. For example, GPR40 and GPR120 act as sensors for medium- and long-chain fatty acids (FAs) in both primary CCK-producing cells [3] and the murine enteroendocrine cell line STC-1 [4]. Data on the expression and functions of taste receptor type-2 family members [5, 6], Toll-like receptors [7, 8], and transient receptor potential channels [9, 10] have also been reported. These sensors are thought to play an important role in preventing the absorption of potentially toxic compounds through CCK-mediated gut motility and secretions [6, 8].

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Abbreviations: AITC, allyl isothiocyanate; Alcs, alcohols; Alds, aldehydes; CCK, cholecystokinin; LDH, lactate dehydrogenase; TRPA1, transient receptor potential ankyrin 1

FAs are potent stimuli of CCK secretion [4, 11], and chain length dependency has been determined both in vivo [12] and in vitro [11]. In contrast, though they are not typical nutrients, the CCK-releasing activities of other aliphatic compounds (aldehydes; Alds and alcohols; Alcs) are still unclear. Unsaturated Alds are generated from heated oil and lipid oxidation, and cause oxidative stress [13, 14].

Previous studies reported the role of the transient receptor potential ankyrin 1 (TRPA1) channel as a sensor for an unsaturated Ald in TRPA1 overexpressing HEK 293 cells and neuronal cells [15–17]. Additionally, the functional expression of TRPA1 has been demonstrated in CCK-producing STC-1 cells [10]. Allyl isothiocyanate (AITC) is known to be a pungent component in mustard oil and wasabi (Japanese horseradish), and PUFAs induce CCK secretion via TRPA1 [10, 18]. Based on these data, we hypothesized that unsaturated Alds are capable of stimulating CCK secretion, possibly through TRPA1.

In the present study, we explored the relationship between carbon chain structure (chain length and unsaturation degree) and the CCK-releasing activity of various types of aliphatic Alds in CCK-producing enteroendocrine cells. In addition, we examined the involvement of the TRPA1 channel in Ald-induced CCK secretion and intracellular calcium mobilization.

2 Materials and methods

2.1 Materials

Cell culture consumables (DMEM, fetal bovine serum, penicillin/streptomycin, and trypsin-EDTA solution) were purchased from Invitrogen (Carlsbad, CA). Fura-2-AM and Pluronic F-127 were obtained from Molecular Probes (Leiden, Netherlands). Poly-L-lysine solution (0.1%), HEPES, ruthenium red, *O,O'*-bis(2-aminoethyl)ethyleneglycol-*N,N,N',N'*-tetraacetic acid (EGTA), and HC-030031 were all purchased from Sigma (St. Louis, MO). The Alds, Alcs, and FAs used in this study are listed in Supporting Information Table 1. Unless specified, all other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2 Cell culture

STC-1 cells (a gift from Dr. Hanahan, University of California, San Francisco, CA) were grown in DMEM (Invitrogen, Cat. No. 12100–038) supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 500 µg/mL of streptomycin. The cells were incubated in a humidified, 5% CO₂ atmosphere at 37°C. Cells were routinely subcultured by trypsinization upon reaching 80–90% confluency. The cells at passage numbers of 20–30 were used for experiments. Each experiment was performed by using the cells with an identical passage number.

2.3 CCK secretion study in STC-1 cells

STC-1 cells were seeded in 48-well culture plates at a density of 1.25×10^5 cells/well and grown for 2–3 days until reaching 80–90% confluency. Cells were washed twice with HEPES buffer to remove the culture medium and then exposed to the test agents (dissolved in HEPES buffer) for 60 min at 37°C. The HEPES buffer (pH 7.4) was composed of the following: 140 mM NaCl, 4.5 mM KCl, 20 mM HEPES, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 10 mM D-glucose, and 0.1% BSA. Following incubation, the supernatants were collected and centrifuged at $800 \times g$ for 5 min at 4°C to remove the remaining cells. The supernatants were then stored at –50°C until the CCK concentration was measured using a commercially available enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Belmont, CA). The primary antiserum provided in this kit cross-reacts (100%) with sulfated and nonsulfated CCK (26–33), CCK-33 (porcine), caerulein, gastrin-1 (human), and big gastrin-1 (human). The antiserum also cross-reacts (12.6%) with CCK (30–33); however, 0% cross-reaction with pancreatic polypeptide (human) and vasoactive intestinal peptide (including human, porcine, and rat). Because STC-1 cells do not express detectable levels of gastrin [19], we selected an EIA kit in which the antibody cross-reacts with gastrin. The coefficients of the intra- and interassay variation were <5% and <14%, respectively. The results of the CCK secretion experiments were expressed as a percentage relative to the blank or vehicle control treatments.

2.4 Measurement of cytotoxicity in STC-1 cells

Cytotoxic effects on STC-1 cells were determined by measuring the release of lactate dehydrogenase (LDH) into the supernatant of STC-1 cells exposed to test agents, as described above. The measurement of LDH was performed using a cytotoxicity detection kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Cytotoxicity was calculated as the relative release (%) of LDH after exposure to the test agents compared to the total LDH (100%) released upon treatment with lysis reagent.

2.5 Intracellular Ca²⁺ measurement in STC-1 cells

To measure intracellular Ca²⁺ concentrations ([Ca²⁺]_i), cells were grown on 0.025% poly-L-lysine-coated coverslips (1.3 cm²) at a density of $3\text{--}5 \times 10^5$ cells/well in 24-well plates, and used 24–48 h after seeding. The cytoplasmic [Ca²⁺] in cells grown on coverslips was determined using a dual-excitation spectrofluorophotometer (CAF-110; JASCO, Tokyo, Japan) with the Ca²⁺ sensitive-ratiometric dye, Fura-2-AM. Cells cultured on a coverslip were loaded with 2 µM Fura-2-AM dissolved in HEPES buffer containing 0.005% Pluronic F-127 and incubated at 37°C for 20 min. The pH of all buffers was adjusted to 7.4. After loading the Fura-2-AM, the coverslip

was mounted into the folder and washed with HEPES buffer. The folder was then inserted into the cuvette of the spectrofluorophotometer, and the experimental liquid was continuously stirred in the cuvette at 1000 rpm at 25°C. Fluorescent intensities were measured at an emission wavelength of 510 nm and excitation wavelengths of 340 and 380 nm with 10 s intervals. After stabilizing basal fluorescence, the cells were exposed to the test agents. The data are expressed as changes in the fluorescence ratio ($\Delta 340/380$ nm) from the basal levels before exposure to the test agents (0 min). Cell viability was assessed by exposing the cells to 40 mM KCl after a challenge with the test agents.

2.6 Statistical analyses

The results are expressed as the mean \pm SEM. Statistical significance was assessed using a one-way ANOVA, and significant differences relative to the control treatments were determined using Dunnett's post-hoc test.

3 Results

3.1 Di-unsaturated aldehydes potently stimulate CCK secretion from STC-1 cells

We first explored the CCK release in response to three types of aliphatic compounds (i.e. hexanal, 1-octen-3-ol, and *trans,trans*-2,4-decadienal). Of these, *trans,trans*-2,4-decadienal (*t,t*-2,4-C10) induced a significant increase in CCK secretion at 100 μ M (Fig. 1A) as same as β -conglycinin peptone (β conP), a positive control, which has been shown to stimulate CCK secretion in in vitro and in vivo [20, 21]. Based on this result, we further examined various Alds having different chain lengths and degrees of unsaturation.

Saturated Alds (C4, C5, C7-C13) did not induce CCK secretion (Fig. 1B–D). Among the mono-unsaturated Alds, 2-C3, *t*-2-C4, and *t*-2-C7 significantly increased CCK secretion (Fig. 1B), and their CCK-releasing activity tended to decrease as their carbon chain length increased (Figs. 1B–D, 2A and B). Although the increment of CCK release induced by *t*-2-C6 was insignificant when analyzed with multiple comparison test (Dunnett's test), the *p*-value calculated by Student's *t*-test in comparison to vehicle control was less than 0.05. In another experiment (Figs. 2B and 5A), *t*-2-C6 Ald induced significant CCK secretion. These results suggest the Ald has CCK-releasing effect, but the effect is not so potent in our experimental condition. All of the di-unsaturated Alds tested induced significant CCK secretion, independent of their chain length. Dose-dependent CCK release was observed by potent di-unsaturated Alds (*t,t*-2,4-C6 and *t,t*-2,4-C10), and *t,t*-2,4-C10 induced significant CCK secretion at 50 μ M (Fig. 1E).

3.2 Aldehydes induced greater CCK release compared to alcohols and FAs

We compared several potent Alds with Alcs and FA of identical chain lengths and degrees of unsaturation. Regardless of functional group, saturated compounds (C6 and C10) did not induce CCK secretion (Fig. 2B and C). Mono-unsaturated (2-C3, *t*-2-C6, and *t*-2-C8 but not *c*-4-C10) and di-unsaturated (*t,t*-2,4-C6 and *t,t*-2,4-C10) Alds induced a significant increase in CCK secretion (Fig. 2A–C). With the exception of 2-C3 FA (Fig. 2B) and *t,t*-2,4-C10 Alc (Fig. 2C), the majority of the tested Alcs and FAs failed to induce CCK secretion. Although 2-C3 FA (Fig. 2B) and *t,t*-2,4-C10 Alc (Fig. 2C) induced significant CCK secretion, their potencies were lower than Alds with identical carbon chain structures. We did not include *t,t*-2,4-C10 FA in the present study because it was not commercially available.

3.3 LDH-release from STC-1 cells

To examine whether CCK release is caused by Alds-induced cell lysis, LDH-release was measured. All of the tested functional groups did not induce LDH-release, regardless of carbon chain length or their degree of unsaturation (Fig. 3)

3.4 Unsaturated aldehydes induced intracellular calcium mobilization

Intracellular calcium mobilization induced by FAs [19, 22], dietary peptides [23, 24], and bombesin [25] triggers CCK secretion in enteroendocrine cells. We examined whether unsaturated Alds also induce an increase in intracellular calcium concentration in STC-1 cells. Propenal (2-C3) rapidly and most potently increased $[Ca^{2+}]_i$ relative to other unsaturated Alds (Fig. 4A and D). As the carbon chain length of *trans*-2 unsaturated Ald increased, the maximum change in intracellular calcium gradually decreased (Fig. 4A and B). *Trans*-4 unsaturated Alds had no ability to induce an increase in $[Ca^{2+}]_i$ (Fig. 4D). Di-unsaturated Alds also induced a sustained increase in $[Ca^{2+}]_i$ (Fig. 4C). In contrast to mono-unsaturated Ald, the maximum change in $[Ca^{2+}]_i$ was gradually increased as the carbon chain length of di-unsaturated Alds increased.

We next compared the ability to induce intracellular calcium signaling among Alds, Alcs, and FAs (C6-saturated and C6-unsaturated). Mono-unsaturated (*t*-2-C6) and di-unsaturated (*t,t*-2,4-C6) Alds clearly induced an increase in calcium mobilization; however, saturated C6 Ald induced only a minor increase (Fig. 4E). Neither the C6-Alcs nor the C6-FAs induced calcium mobilization, regardless of their degree of unsaturation (data not shown).

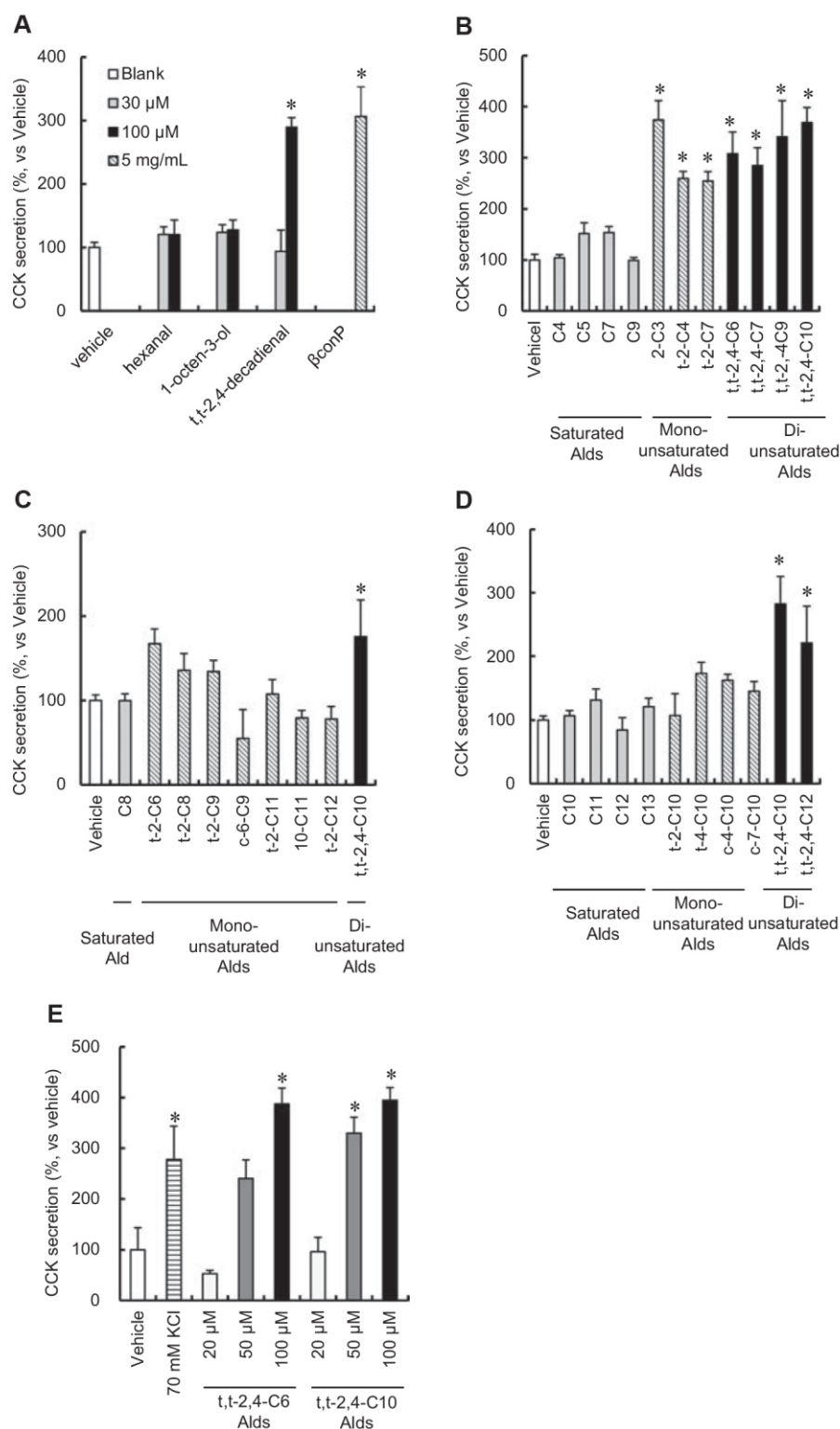


Figure 1. STC-1 cells cultured in 48-well plates were exposed to (A) three aliphatic compounds (30 and 100 μ M) and 5 mg/mL protein hydrolysate (β -conglycinin peptone: β conP) for 60 min. To compare the effect of various AlDs, saturated, mono-, and di-unsaturated AlDs (100 μ M) were tested (B–D). Active compounds (*t,t*-2,4-C6 and *t,t*-2,4-C10) were treated for 60 min in dose dependently (20–100 μ M) (E). These experiments (A–E) were independently performed. CCK concentrations in the supernatant were measured using EIA. The values represent the relative CCK concentration in the supernatant, and are expressed as the mean \pm SEM of 4–8 wells. Significant differences ($p < 0.05$, Dunnett's test) relative to the vehicle are indicated with an asterisk (*).

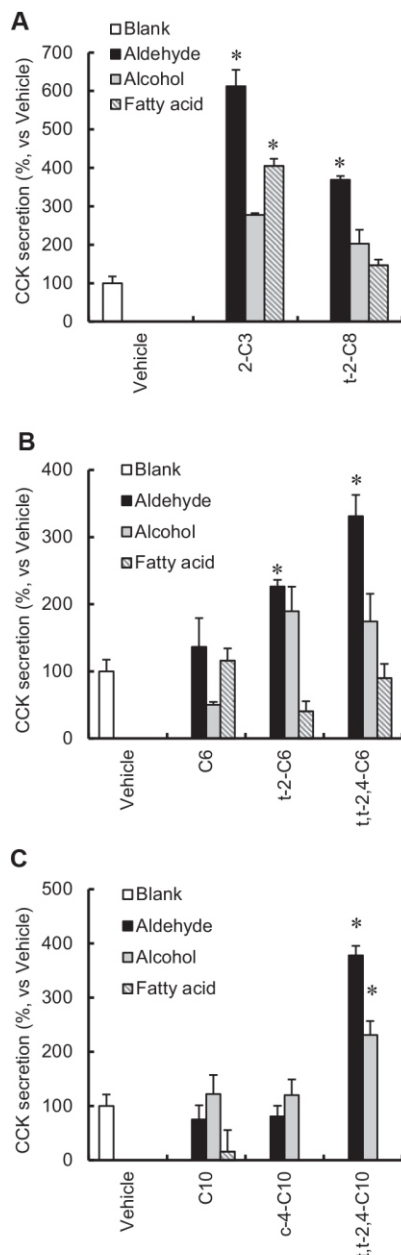


Figure 2. STC-1 cells cultured in 48-well plates were exposed to various Alds, Alcs, and FAs (100 μ M) of identical carbon chain structure (i.e. length and unsaturation degree). (A) 2-C3, *t*-2-C8, (B) saturated/unsaturated C6, and (C) saturated/unsaturated C10 were compared. CCK concentrations in the supernatant were measured using EIA. The values are the relative CCK concentrations in the supernatant expressed as the mean \pm SEM of 3–6 wells. Significant differences ($p < 0.05$, Dunnett's test) relative to the vehicle are indicated with an asterisk (*).

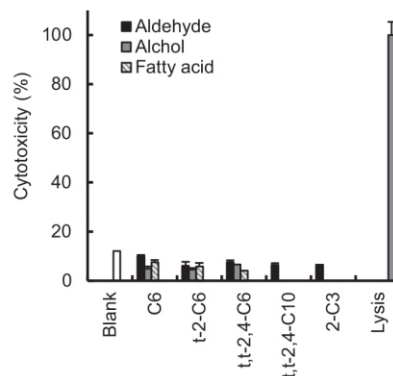


Figure 3. LDH activity was measured in the supernatant of STC-1 cells exposed to Alds, Alcs, or FAs. For the high LDH activity control, a lysis reagent was used to release all intracellular LDH. The values represent LDH activity (%) relative to the high LDH control and are expressed as the mean \pm SEM of three wells.

3.5 Involvement of extracellular calcium in unsaturated aldehyde-induced CCK secretion and intracellular calcium mobilization

Extracellular calcium influx is one of the main pathways involved in CCK secretion in STC-1 cells [1]. Because unsaturated Alds induced intracellular calcium mobilization (Fig. 4), we examined the involvement of extracellular calcium in unsaturated Ald-induced CCK secretion and intracellular calcium mobilization. Basal CCK secretion by HEPES buffer containing 0.1% EtOH was not affected by the presence or absence of extracellular calcium concentrations. CCK secretion induced by unsaturated Alds was abolished in the absence of extracellular calcium regardless of their carbon chain length and degree of unsaturation (Fig. 5A). In the absence of extracellular calcium, *t,t*-2,4-C10 failed to induce calcium mobilization (Fig. 5B).

3.6 Unsaturated aldehydes induced CCK secretion via TRPA1

AITC, a TRPA1 agonist, has been shown to induce CCK secretion and calcium signaling in STC-1 cells [10, 26], and some of Alds are known to be TRPA1 agonists [16]. Therefore, we examined whether TRPA1 is involved in unsaturated Ald-induced CCK secretion. AITC-induced CCK secretion was expectedly abolished by a TRPA1 selective inhibitor, HC-030031. CCK secretion induced by *t,t*-2,4-C10 was largely inhibited by HC-030031, and secretion by 2-C3 was partially inhibited by HC-030031 (Fig. 6A). The TRPA1 inhibitor had no effect on denatonium benzoate-induced (a bitter taste receptor agonist) CCK secretion (Fig. 6A). In addition, calcium mobilization induced by *t,t*-2,4-C10 was largely suppressed by the TRPA1 inhibitor (Fig. 6B).

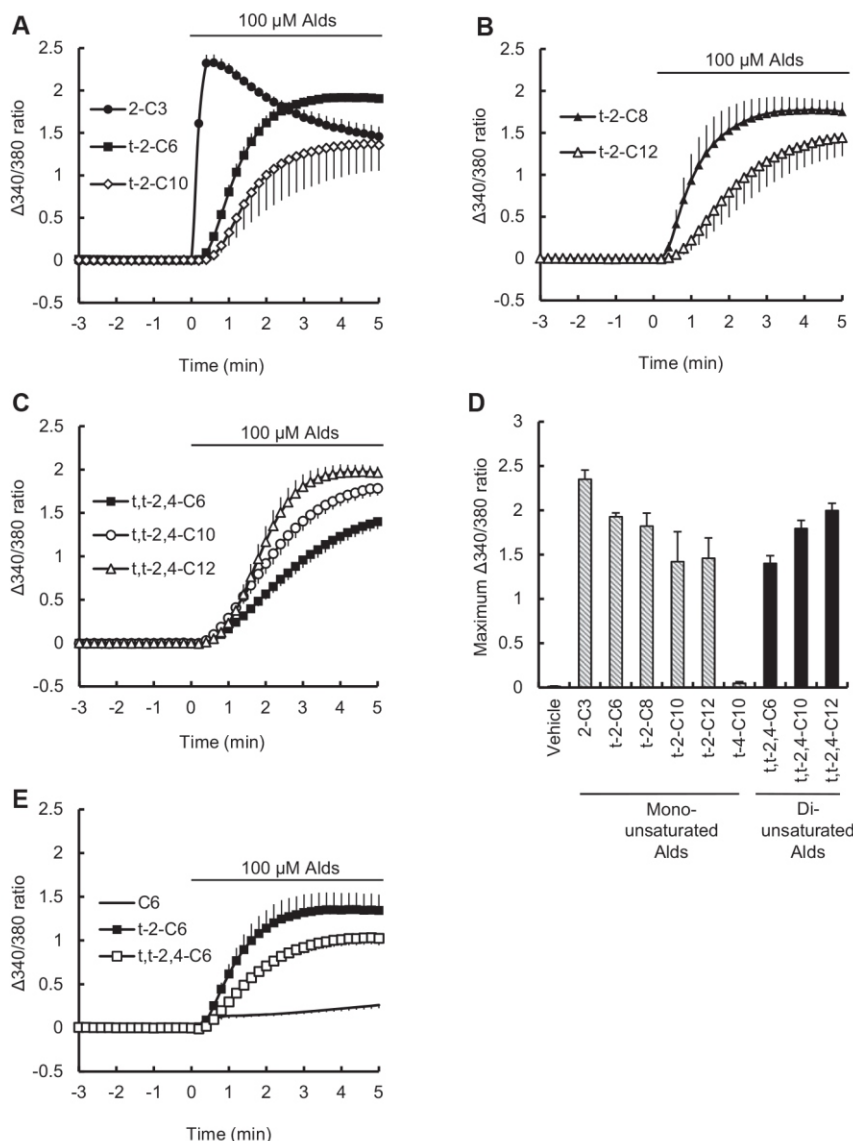


Figure 4. STC-1 cells cultured on coverslips were loaded with Fura-2-AM, and intracellular Ca^{2+} concentrations were measured ratiometrically using a spectrofluorometer. Cells were exposed to 100 μ M of various Alds (A–D) and 100 μ M of saturated/unsaturated C6 (E) in HEPES buffer. The data are expressed as changes from the basal state (0 min) ($\Delta 340/380$ ratio) (A–C, E) or maximum changes in the $\Delta 340/380$ ratio (D). The values represent the mean \pm SEM of four experiments.

4 Discussion

The present results demonstrate that mono- and di-unsaturated Alds, but not saturated Alds, potentially stimulate CCK secretion in the murine enteroendocrine cell line STC-1. Because Alcs and FAs with identical carbon chain structures (chain length and degree of unsaturation) did not have the same effect, the Ald group is hypothesized to be responsible for the CCK-releasing activity. These results reveal the potent CCK-releasing activity of unsaturated Alds. In addition, the role of TRPA1 as the sensor of Alds is suggested by the present study.

Structure–activity relationship studies determined that the degree of unsaturation is an important feature for Alds to

stimulate CCK secretion. This was clearly demonstrated in Figs. 1 and 2 as the CCK secretory responses were greater after the di-unsaturated Alds treatments compared to mono-unsaturated Alds treatments (i.e. C6, C9, C10, C12). However, for mono-unsaturated Alds, the tendency for reverse chain length dependency was observed. Relative increments of CCK release induced by mono-unsaturated aldehydes (*t*-2-C6 and *t*-2-C8) varied among the experiments (Figs. 1C, 2A, B, and 5A) though that reason was unknown. Therefore, we focused on di-unsaturated aldehyde especially *t,t*-2,4-C10, which stably stimulated CCK secretion in following experiments. In contrast to the *trans*-type Alds, none of the *cis*-type Alds induced significant CCK secretion, suggesting that CCK-producing cells are relatively sensitive to the *trans*-structure.

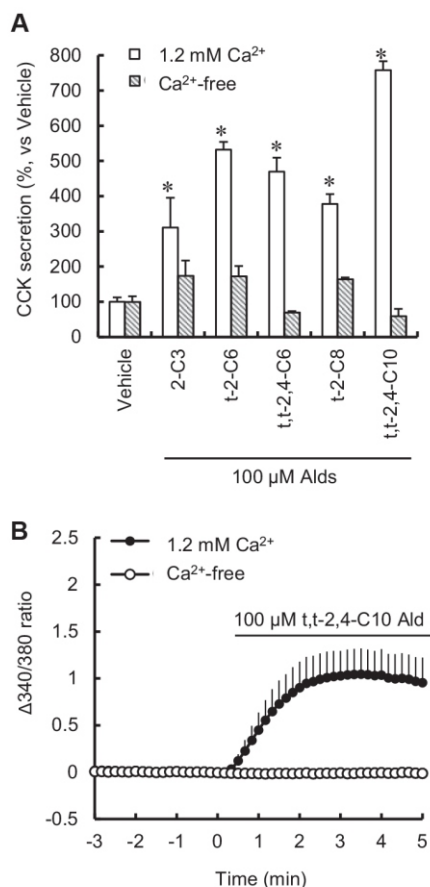


Figure 5. (A) STC-1 cells cultured in 48-well plates were exposed to 2-C3, *t*-2-C6, *t,t*-2,4-C6, *t*-2-C8, or *t,t*-2,4-C10 (100 μM) for 60 min with or without extracellular calcium. CCK concentrations in the supernatant were measured by EIA. The values represent the mean ± SEM of 3–4 wells. Significant differences ($p < 0.05$, Dunnett's test) relative to vehicle treatment (0.1% EtOH) are indicated with an asterisk (*). (B) STC-1 cells cultured on coverslips were loaded with Fura-2-AM and intracellular Ca²⁺ concentrations were measured ratiometrically using a spectrofluorometer. Cells were exposed to 100 μM *t,t*-2,4-C10 with or without extracellular calcium. The data are expressed as changes from the basal state (0 min) (Δ340/380 ratio). The values represent the mean ± SEM of three experiments.

Trans-mono-Alds and *trans,trans*-di-Alds are major volatiles made from fats/oils during heating [13]. It would be interesting to compare these to other di-unsaturated Alds with *cis,cis* or *trans,cis*-structures to clarify the correlation between the *cis/trans* combination in Alds and their CCK-releasing activity. Previous studies on the effect of C18 PUFA on CCK-producing cells demonstrated that the position of the double bond is important for the CCK secretion activity [27]. Although we did not include di-unsaturated Alds with a double bond at the separated position, the present study at least

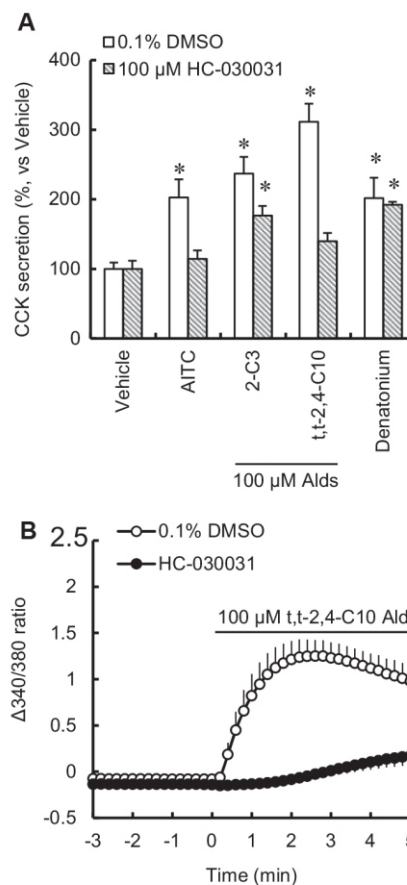


Figure 6. (A) STC-1 cells cultured in 48-well plates were exposed to 100 μM 2-C3, *t,t*-2,4-C10, AITC, or 10 mM denatonium benzoate for 60 min after a 30 min pretreatment with 100 μM HC-030031 or its vehicle (0.1% DMSO). CCK concentrations in the supernatant were measured by EIA. The values represent the mean ± SEM of 4–8 wells. Significant differences ($p < 0.05$, Dunnett's test) relative to vehicle treatment (0.1% EtOH) are indicated with an asterisk (*). (B) STC-1 cells cultured on coverslips were loaded with Fura-2-AM, and intracellular Ca²⁺ concentrations were measured ratiometrically using a spectrofluorometer. Cells were exposed to 100 μM *t,t*-2,4-C10 after a 30 min pretreatment with 100 μM HC-030031 or its vehicle (0.1% DMSO). The data are expressed as the changes from basal state (0 min) (Δ340/380 ratio). The values represent the mean ± SEM of four experiments.

demonstrated that Alds with conjugated dienes have potent CCK-releasing activity.

It has been determined that Alds increase intracellular calcium in a variety of cell types [28, 29]. In the present study, mono- and di-unsaturated Alds, but not saturated Alds (Fig. 4), induced an increase in intracellular calcium concentration. Both intracellular calcium mobilization and CCK secretion induced by unsaturated Alds were abolished in the absence of extracellular calcium (Fig. 5). These results suggest that unsaturated Alds induce CCK secretion through the calcium signaling pathway activated by calcium influx.

However, the degree of calcium mobilization and CCK release induced by mono- and di-Alds is not simply correlated. Indeed, mono- and di-unsaturated Alds induced intracellular calcium mobilization with similar degree; however, CCK-releasing activity was higher in di-unsaturated Alds than mono-unsaturated Alds (C6-C12). Di-unsaturated Alds might induce CCK secretion not only through calcium signaling but also through other pathways.

To examine the specificity of Alds, we compared Alds to Alcs and FAs having identical degrees of unsaturation and carbon chain lengths. As shown in Figs. 2 and 4, unsaturated Alds, rather than other functional groups (Alcs and FAs), strongly stimulated CCK secretion and intracellular calcium mobilization regardless of their carbon chain length. These results demonstrate the importance of the Ald group as the key feature that is necessary to induce CCK secretion and intracellular calcium mobilization, in addition to their degree of unsaturation. In previous studies, saturated FAs induced CCK secretion at $>100\ \mu\text{M}$ (C13, C14) and $>500\ \mu\text{M}$ (C11, C12) [19], and unsaturated FAs (C16, C18, C20, C22) have also been shown to induce CCK secretion at $100\ \mu\text{M}$ [4, 27]. Our results showed that hexanoic acid (C6 FA) failed to stimulate CCK secretion and intracellular calcium mobilization though FAs induce intracellular calcium signaling in STC-1 cells in general [19, 22]. A recent study has demonstrated that pentanoic acid (C5 FA), similar aliphatic compound to C6 FA, stimulated PYY secretion from STC-1 cells [30], suggesting that FAs-induced intracellular calcium mobilization may not be necessarily suitable indicator of one specific satiety hormone release from STC-1 cells. At least, FAs may require long chain lengths and higher concentrations to trigger significant CCK secretion. Some of test compounds tended to reduce CCK secretion compared to vehicle treatment. Suppressive effect of galanin (a peptide hormone) on CCK secretion had been demonstrated in a previous paper [31]. Although the mechanism is unclear, those compounds might affect such suppressive pathway.

To consider an Ald sensing mechanism, we investigated the involvement of one of the transient receptor potential superfamily channels, TRPA1. TRPA1 is an excitatory ion channel targeted by environmental irritants, including AITC, short chain Alds (propenal and *trans*-2-pentenal) [16]. Alcs also function as TRPA1 agonist although effective concentration of Alcs is higher than that of Alds [32]. In STC-1 cells and human duodenum, the expression of TRPA1 has been demonstrated [10]. It was demonstrated that AITC increased intracellular calcium concentration [26] and stimulated CCK secretion through TRPA1 in STC-1 cells [10]. CCK secretion was abolished in the absence of extracellular calcium, which implies that calcium influx from extracellular is important for TRPA1-mediated CCK secretion [10]. In the present study, intracellular calcium mobilization and CCK secretion induced by *t,t*-2,4-C10 were both largely reduced by a selective TRPA1 antagonist and by the removal of extracellular calcium. Although the TRPA1 antagonist only partially attenuated 2-C3-induced CCK secretion, these results suggest that TRPA1

functions as a sensor for unsaturated Alds to release CCK secretion in CCK-producing enteroendocrine cells. Further investigations are necessary to elucidate the effect of Ald on CCK secretion and the function of TRPA1 in CCK-producing cells in *in vivo*.

Unsaturated Alds are found in deteriorated oils, derived from PUFAs, and known to be potent factors in oxidative stress [33, 34]. The concentrations of *t,t*-2,4-C10 in food products and odorant have been shown previously [35, 36]. Various odor Alds from stewed beef vegetable gravy and pork vegetable gravy are detected at the level of $\mu\text{g/kg}$ [35]. Andrikopoulos et al. reported that the concentrations of *t,t*-2,4-C10 in various dietary flying oil were higher than odorant, its level reached to mg/kg [36] that is approximately equivalent to $100\ \mu\text{M}$ ($15.2\ \text{mg/L}$). Recently, it has been reported that PUFAs induce CCK secretion in STC-1 cells through the activation of TRPA1 [18]. Our results demonstrated that TRPA1 is also involved in unsaturated Ald-induced CCK secretion. CCK is involved in induction of nausea by lipid ingestion [37, 38]. The finding in the present study may explain the mechanism for induction of nausea by deteriorated lipid ingestion. TRPA1 functions not only in CCK-producing cells but also in other serotonin-producing enteroendocrine cells (e.g. enterochromaffin cells) to regulate gastrointestinal motility [39, 40]. These findings suggest that CCK-producing cells, as well as serotonin-producing cells, recognize unsaturated Alds to prevent the excess absorption and ingestion of deteriorated oils by delaying gastric emptying and inducing appetite suppression. TRPA1 possibly act as the sensor for both nutrients (PUFAs) and xenobiotic compounds (e.g. Alds) in CCK-producing cells. This hypothesis is supported by previous reports that demonstrate the function of TLR4 (where TLR is Toll-like receptor) in CCK-producing cells [7, 8]. Further, it should be clear whether TRPA1 is expressed in human CCK-producing cells in future studies for reveal the TRPA1 functions in physiological situation in human.

In summary, we found that unsaturated Alds are potent stimuli for CCK secretion in enteroendocrine cells. It was also found that the Ald group, but not the Alc or carboxyl groups, is required for activating CCK release and intracellular calcium signaling. CCK secretion and intracellular calcium mobilization induced by unsaturated Alds were attenuated by a TRPA1 antagonist. These results demonstrate that unsaturated Alds stimulate CCK secretion via TRPA1 in CCK-producing cells.

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A Comprehensive Review of Characterization and Detection of Adulteration of Extra Virgin Olive Oil

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Abstract

Determination of authenticity of extra virgin olive oils has become very important in recent years due to the increasing public concerns about possible adulterations with relatively cheap vegetable oils and deodorized olive oils. The analysis and authentication of extra virgin olive oil (EVOO) represent very challenging analytical chemical problems. Each country may have its own definition of olive oil and its own limits for particular oil parameters and monitoring guidelines in International Standards. Generally, these standards are based on the International Olive Council (IOC) and Codex Standards but often there are alternatives for different countries. The aim of this review is to provide specific information about olive oils with special concern about extra virgin olive oil (EVOO) and analytical techniques to detect adulteration of EVOO. The guidelines for genuine olive oil are adapted from the IOC, other legitimate and appropriate sources. The contents provide information on possible adulterants in olive oil, the underlying causes of adulteration, and how to test for the presence of these adulterants.

Keywords: Extra Virgin Olive Oil; Detection of Adulteration; International Standards

Introduction

Olive is the fruit of the olive tree (*Olea europaea*, *Oleaceae*). The fruit is an oval-shaped drupe composed of a pericarp (skin and flesh) and the endocarp (seed or pit). The seed contributes 15-30% of the weight of the olive depending on the cultivar. The olive contains around 50% water and 20% oil, although these proportions vary widely among olive cultivars and the environmental conditions in which they are grown. There are many cultivars grown worldwide and although around 600 can be found in the literature, the precise number is unknown. IOC has published a World catalogue of Olive Varieties describing 139 cultivars grown across 23 countries, which IOC claims represent almost 85% of the total world's acreage of olives [1].

Olive oil: Olive oil is the oil fraction of the olive fruit, i.e., pulp and seed. It is composed basically of triacylglycerols (triglycerides) with the predominant fatty acid being oleic acid (55-83%). The other major fatty acids include palmitic (7.5-20%), stearic (0.5-5%) and linoleic (3.5-21%). The oil contains some minor compounds including phytosterols, aliphatic alcohols, pigments, and a range of more polar phenolic compounds including hydroxytyrosol and tyrosol. Refined olive oils have considerably reduced levels of these minor compounds [1].

Pomace: Pomace is the solid waste material left after olive oil and water have been extracted by pressing or centrifugation of olives. Generally, the pomace contains 5-10% of the oil after

centrifugation and may be further extracted using solvents [1].

A detailed description of the various olive oil grades is provided as following [1]:

Virgin (or natural) olive oil

The initial division is based on extraction methods used and oil is classified as "virgin" olive oil when it is extracted from the fruit by mechanical means, in a way which will not alter the oil characteristics. This process excludes oils obtained using solvents or re-esterification processes and of any mixture with oils of any other kind or source. Modern methods of extraction of virgin oil involve washing, grinding or crushing the fruit, mixing or malaxing the paste, and then centrifuging to separate the oil from the pomace. This category, virgin olive oil, can be further divided, based on oil quality, into the following four groups:

1. *Extra virgin olive oil* (EVOO) must meet chemical and organoleptic limits. In particular, the free fatty acids (measured as oleic acid) must be less than, or equal to 0.8% w/w. In addition, the sensory requirements of this grade require that the oil have zero "defects" and positive attributes of fruitiness in excess of zero, based on sensory assessment.

2. *Virgin olive oil* must have less than, or equal to 2.0% w/w free fatty acids. The sensory requirements are that the oil be between 0 to 3.5 defects, as described by the IOC and greater than zero for fruitiness.

3. *Ordinary virgin olive oil* is a category used by IOC but not all countries use this grade. It describes virgin olive oil with a free fatty acid level of not more than 3.3% w/w.

4. *Lampante virgin olive oil*, also referred as “crude” in some standards, refers to any virgin olive oil exceeding the free fatty acid limits discussed above or has a peroxide value (a measure of vegetable oil rancidity) in excess of 20 millequivalents (mEq) peroxide oxygen per kg/oil per the IOC standard, while California’s standard lowers this value to 15. This oil is considered unfit for human consumption in this state and requires refining or is otherwise used for industrial purposes. All grades of edible virgin olive oil should have a peroxide value of less than, or equal to 20 mEq peroxide oxygen per kg/oil. For lampante oil, there is no limit for peroxide value.

Refined olive oil

- *Refined olive oil*, sometimes called ‘Pure Olive Oil’, is olive oil suitable for consumption after it goes through a food grade refining process. Any process structure of the oil. Due to refining, the free fatty acids content is low and for this grade must be no more than 0.3% by weight.

- *Olive oil* composed of refined olive oil and virgin olive oil, is a blend of refined olive oil and virgin olive oil (other than lampante oil). In this case the free fatty acid must be not more than 1.0% by weight.

Olive pomace oil

- *Crude olive pomace oil* is the oil extracted, using solvents such as hexane, from the solid waste, after producing virgin olive oil. The oil requires refining if used for human consumption. Otherwise it is used for industrial purposes.

- *Refined olive pomace oil* is obtained by refining crude olive pomace oil in a way that does not change the glyceridic structure of the oil. The free fatty acid concentration must not exceed 0.3% by weight.

- *Olive pomace oil composed of refined olive pomace oil and virgin olive oils*. As the name implies, this grade of oil is a blend of virgin olive oil and refined olive pomace oil. The free fatty acid content should not exceed 1.0% by weight.

Olive Oil Consumption Set to Outpace Production for a Change

Worldwide olive oil production will be somewhat lower than consumption in the 2020 season. With current estimates suggesting production of around 3.11 million tons of olive oil and 3.14 million tons expected to be consumed, there could finally be some good news for producers, according to the international consultant Juan Vilar. “We expect that during this campaign, consumption will be higher than production” Vilar said at a recent Andalusian webinar about olive production, according to the Spanish news agency COPE. “In addition, price increases have been seen in all categories and this shows that although a change in strategy is necessary, it is a

ball of oxygen for the traditional olive grove, which accounts for 70 percent of the harvest and that, without a doubt, has lived through some difficult times.” There is also a growing role around the world for the “modern olive grove,” said Vilar, which now accounts for “40 percent of all olive oil produced”—numbers that reveal “a trending change and a reality which is constantly growing.” Vilar also noted how the growing popularity of olive farming in five continents “brought to a total of 11.5 million hectares dedicated to olive trees.” Those numbers pushed worldwide production to exceed 3 million tons “feeding households in more than 180 countries.” [2].

Adulteration of food

Adulteration of food worldwide is a major issue for health and safety. Numerous cases of food poisoning and health issues have been reported through mislabeling of food or blend [1].

Sources of information supporting confirmation of adulteration

The adulteration of olive oil is not a new phenomenon; texts describing the substitution of olive oil with oils from poppy (*Papaver somniferum*, *Papaveraceae*) seeds, peanuts, sesame (*Sesamum indicum*, *Pedaliaceae*) seeds, or beech (*Fagus sylvatica*, *Fagaceae*) nuts date back to the 19th century.

In 1981, the death of more than 600 people in Spain was attributed to the consumption of a product sold as olive oil. Investigations led to the belief that it was actually canola seed oil which had been denatured by the addition of aniline to make it suitable for industrial use. Fraudulent operators had tried to refine the oil and had sold it as olive oil.

In 2007, an article on the olive oil trade in *The New Yorker* magazine described a shipping tanker in 1991 in Ordu, Turkey loading several hundred tons of hazelnut oil for which the ship’s official documents listed Greek olive oil. It was delivered to an Italian olive-oil producer and sold as olive oil.

A major study from the University of California, Davis in 2010 illustrates the degree of fraudulent olive oil sales in the United States, the world’s largest importer of olive oil. From 14 imported brands and five Californian brands of EVOO, purchased from retail stores in three regions of California, a total of 69% of imported olive oil samples and 10% of California olive oil samples, labeled as EVOO, failed to meet the IOC and California Olive Oil Council sensory (organoleptic) standards for EVOO. Many of these also failed either IOC ultraviolet (UV) tests and/or pyropheophytin (PPP) and diacylglycerols (DAG) limits. The report was questioned by various importers in defense of their product, and the study was repeated with more stringent controls. In this study, five imported brands were sampled from each of 18 retail outlets. Of the five top-selling imported “extra virgin” olive oil brands in the United States, 66 of 90 of the samples analyzed again failed the IOC sensory standards for EVOOs analyzed by two IOC accredited sensory panels. Although EVOO deteriorates

in store shelves if they are subject to light or have been exposed to high temperature, such as transportation in midsummer, it is expected that the oil will be EVOO quality when consumed.

There is an abundance of published reports on olive oil adulteration over the past decade, which is often covered by the mainstream media based on evidence provided after olive oil analysis and subsequent regulatory actions by government authorities. The *Olive Oil Times*, a digital news outlet covering the olive oil sector, has an entire section devoted to olive oil fraud. Most of these cases involve the sale of lower grade olive oil as EVOO or virgin olive oil, or misrepresenting the country of origin. Other articles have reported on the sale of olive oil mixed with lowercost vegetable oils, sometimes with the addition of chlorophyll, β -carotene, or other unidentified dyes.

In 2018, the Spanish company Dcoop was accused of blending canola and olive oil and selling it in the United States as Pompeian® brand. The label on Pompeian OlivExtra Original bottles described the oil as “composed of 85% canola oil and “First Cold Press Extra Virgin Oil”. However, it is reported that the oil contained almost all Canadian canola oil with less than 1% being EVOO.

In April 2018, it was reported that Deoleo USA had paid seven million US dollars to settle a class action in California. Deoleo had allegedly mislabeled Bertolli® olive oil as “Imported from Italy” whereas Deoleo imported olive oil from several countries including Morocco, Tunisia, Greece, and Turkey. Additionally, the oil failed to meet standards limit for EVOO up to the “best by” date.

Known adulterants:

a. The most valuable grade of olive oil is EVOO. The most common adulterant found in EVOO are inferior grades of virgin or refined olive oil, as these grades are often products which have failed either the chemical or the sensory (organoleptic) standard to meet the standards of the higher-priced EVOO. They may be blended with EVOO and marketed fraudulently as EVOO. Virgin or refined olive oil have a poorer flavor and a much lower level of the antioxidants for which EVOO is renowned and for which it is purchased. Consumers may think they are getting the organoleptic and nutritional benefits of EVOO, but they are actually consuming an inferior product.

b. Other lower-cost virgin oil may also contain refined oil or pomace oil to obtain a greater financial return for the producer. In these cases, the adulterant may contain products from solvent extraction or refining that are not characteristic of olive oil, including traces of hexane solvent or trans fatty acids and glycidyl fatty acid esters. Trans fatty acids and fatty acid esters are generated at high temperature mainly during the deodorization process. Due to the carcinogenic property of glycidol, a tolerance value of 1ppm was recently established in Europe for food grade oils.

c. Seed oils are significantly less expensive than olive oil and are commonly used as adulterants for EVOO or refined olive oil blends. The most common seed oils include canola/rapeseed/colza, sunflower, and soybean oils. The mixture of seed oils and EVOO produce a product that has a different fatty acid profile to that of EVOO. It is also likely to have reduced antioxidants, as seed oils are generally refined.

d. Nut-based oils are also used to adulterate EVOO and refined olive oil blends. In particular, hazelnut oil has been reported to be used in blends with Italian olive oil. Hazelnut oil is a concern for authorities as it is difficult to detect levels of less than 10% in EVOO.

e. The green pigment, chlorophyll, is highest in newly extracted olive oil and gives the oil a fresh green appearance. This color is much lower or non-existent in refined or old oil. Chlorophyll can be chemically modified with copper to produce a pigment with a permanent green color and this can be added to old oil to give a perception of freshness and higher quality. Similarly, the orange pigment β -carotene has been found to be added to olive oil to impart a more desirable color. EVOO has a reasonably long shelf life when compared with other oils and will deteriorate over time as antioxidants are depleted and the peroxide value and free fatty acids increase. The useful life of EVOO can range from 12 to 24 months, depending on quality of the fruit and oil when it is pressed. This oil may be labeled with “use by” dates in excess of the oil life, or the oil may simply be sold as EVOO even though its quality has diminished. The sale of this oil is also fraudulent as it does not provide the health benefits and nutritive value expected of EVOO and declared on the label.

Detection of adulteration of EVOO Analytical Methods and References for EVOO as Described by IOC

A summary of analytical methods and references for EVOO as described by the International Olive Council [3] to determine the grade of olive oil, or possible adulteration, is shown in Table 1.

If a particular fault is perceived, the most relevant tests can be selected. Based on Table 1, those tests may reveal the following findings:

• **Oil produced from poor quality fruit or poor processing conditions:** If the fruit is damaged by disease, frost damage, insects or merely by harsh harvest and storage conditions, there will be an increase in free fatty acids and possibly increased peroxide value. In this case, tests 1, 2, 15 and DAGs would be employed.

• **Poor quality olive oil labeled as EVOO:** Olive oil has to meet certain criteria to be labeled as EVOO. In particular, it must meet the sensory requirements of fruitiness and no defects. It must also be within prescribed limits for free fatty

Tabel 1: Analytical Methods and References for EVOO as Described by the International Olive Council

No	Title
1	Determination of the free acidity according to COI/T.20/Doc. No 34/Rev.1, "Determination of free fatty acids, cold method"
2	Determination of the peroxide value according to COI/T.20/Doc. No 35/Rev.1 "Determination of the peroxide value", ISO 3960, or AOCS Cd 8b-90
3	Determination of the fatty acid composition and <i>trans</i> fatty acid content according to COI/T.20/Doc. No 33/Rev.1: "Determination of fatty acid methyl esters by gas-chromatography"
4	<i>Trans</i> fatty acid content (% <i>trans</i> fatty acids) C18:1 T C18:2 T + C18:3 T according to COI/T.20/Doc. No 33/Rev.1: "Determination of fatty acid methyl esters by gas-chromatography"
5	Determination of the sterol content and alcoholic compounds according to COI/T.20/Doc. No 26/Rev.3: "Determination of the sterol content and alcoholic compounds by capillary gas chromatography"
6	Erythrodiol and uvaol content (% total sterols) according to COI/T.20/Doc. No. 30/Rev. 1: Determination of the composition and content of sterols and triterpene diols by capillary column gas chromatography"
7	Determination of wax content by capillary column, gas-liquid chromatography according to COI/T.20/DOC. 18
8	Determination of the difference between the actual and theoretical ECN 42 triacylglycerol content according to COI/T.20/Doc. No 20/Rev.4, "Determination of the difference between actual and theoretical content of triacylglycerols with ECN 42", or AOCS 5b-89
9	Determination of the stigmastadiene content according to COI/T.20/Doc. No 11/Rev.3, "Determination of stigmastadienes in vegetable oils", or COI/T.20/Doc. no. 16/Rev.1, "Determination of sterenes in refined vegetable oils", or ISO 15788-1 or AOCS Cd 26-96
10	Determination of the content of 2-glyceryl monopalmitate according to COI/T.20/Doc. No 23/Rev.1, "Determination of the percentage of 2-glyceryl monopalmitate" or to ISO 12872
11	Determination of the unsaponifiable matter according to ISO 3596, "Determination of the unsaponifiable matter – Method using diethyl ether extraction", or AOCS Ca 6b-53 or ISO 18609. The results should be expressed in g unsaponifiable matter/kg oil
12	Determination of the content of waxes and alkyl esters according to COI/T.20/Doc. No 28/Rev.2, "Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography"
13	Determination of the organoleptic characteristics according to COI/T.20/Doc. No 15/Rev.10, "Organoleptic assessment of virgin olive oil"
14	Detection of trace metals according to ISO 8294, "Determination of copper, iron and nickel by direct graphite furnace atomic absorption spectrometry"
15	Determination of the absorbency in ultra-violet according to COI/T.20/Doc. No 19/Rev.4, "Spectrophotometric investigation in the ultra-violet", or ISO 3656 or AOCS Ch 5-91

acids, peroxide value and UV absorbency. Therefore, tests 1, 2, 13, 15, as well as PPP and DAGs, would be applied.

- Refined olive oil labeled as virgin olive oil: Oil is refined generally to remove undesirable components such as free fatty acids and peroxides, which are the result of the oil's deterioration. Refining may also be used to remove natural pigments and/or products which may give the crude oil unpleasant flavor or odor, as in the case of crude rapeseed oil. Refining generally has three steps: refining, bleaching, and deodorizing (RBD). These steps, particularly deodorization, involve application of heat to the oil. When oil is heated, there are some common changes which can be detected by testing. Fatty acids, which make up the majority of the oil, can change their configuration from *cis* to *trans* fatty acids. Heating proportions of proportions of PPP and DAG in the oil. To determine the presence of refined oil, tests 4, 9, PPP, and DAG should be used.

- EVOO mixed with seed and nut oils: Seed oils are often refined and therefore tests applied to refined oil would also apply here. However, a more appropriate test would be to measure parameters specific for individual species. For example, the fatty acid composition and the sterol profile are generally good "fingerprints" for specific species. Canola oil, for example, has around 5-10% linolenic acid in the fatty acid profile whereas olive oil has less than 1% by IOC standards. Canola oil also contains a phytosterol named brassicasterol. Brassicasterol is not present in plant species other than those of the genus *Brassica* in the family *Brassicaceae*. A range of tests can be applied to determine the presence of seed or nut oils based on the fatty acid composition and sterols. These include tests 3, 5, 8, 9, 10, 11, and 15.

- Solvent extracted oils including "pomace oil" and seed oils: Solvent extraction, generally with hexane or a similar solvent, is a much more efficient extraction process than merely squeezing or centrifuging the olive paste. However, by

definition, solvent is not permitted in EVOO extraction, and it requires heating to remove any residual solvent traces from the oil. No solvents or extraneous chemicals are permitted in the processing of virgin olive oil. As solvents remove additional compounds from the olive fruit not extracted by regulation methods, analysis for the presence of these compounds can show if solvents have been used. Only traces of wax from the fruit skin are extracted by mechanical means but the wax will dissolve in the extraction solvent. Oils containing excessive amounts of wax are obtained by solvent extraction. The pentacyclic triterpenes erythrodiol and uvaol are extracted from olive pits (seeds), and the presence of these compounds also indicates solvent extraction. Tests to determine solvent extraction include 6 and 7.

• **Old or badly stored olive oil sold as EVOO:** Oil degrades over time, and the by-products are generally similar to that seen in oils that have been heated. Heating causes accelerated aging. Some of the tests used for refined oil can also be used to test for old, or badly stored, oil. Free fatty acids may increase slightly with time. Sensory characteristics will change with a decrease in fruitiness and an increase in defects as the oil becomes rancid. PPP and DAG proportions also change, and these tests are particularly useful to determine aging or poor storage conditions. Tests include 1, 2, 13, 15, PPP, and DAG.

Additional Analytical Methods and References for EVOO as Described by ISO

Pyropheophytin and 1,2-diacylglycerol content: Two methods published by the International Organization for Standardization (ISO) involve the determination of the proportions of PPP and DAG in virgin olive oil. A recent study highlights the value of PPP and DAG indicators of olive oil quality and freshness. There is also a strong correlation with Organoleptic defects. The measurement of pyropheophytins helps detect deodorized olive oils and, together with DAG, is effective in determining oil storage conditions and aging.

International Standards: Each country may have its own definition of olive oil and its own limits for particular oil parameters and monitoring guidelines. Generally, these standards are based on the IOC and Codex standards [4] but often there are alternatives. Individual standards include the following:

• **Codex Alimentarius:** Standard for Olive Oils and Olive Pomace Oils. CXS 33-1981. Adopted in 1981. Revised in 1989, 2003, 2015. Amended in 2009, 2013 [5].

• **European Union:** Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Consolidated version: 04/12/2016 [6].

• **International Olive Council:** Trade Standard. COI/T.15/NC No 3/Rev. 11. 2016 [7].

• **United States:** Standards for Grades of Olive Oil and Olive-Pomace Oil [8].

• **State of California:** Department of Food and Agriculture. Grade and Labeling Standards for Olive Oil, Refined-Olive Oil and Olive-Pomace Oil [9].

• **Australian Standard:** Olive oils and olive-pomace oils. AS 5264—2011 [10].

• **Argentina:** Código Alimentario Argentino. Capítulo VII. Alimentos Grasos. Aceites Alimenticios. Artículos 535 y 536 [11].

• **South Africa:** South African National Standard. Olive oils and olive-pomace oils. SANS 1377:2015 Edition 1 [12].

• **Brazil:** Ministério da Agricultura, Pecuária e Abastecimento. Gabinete do Ministro. Instrução normativa No 1, 30 de Janeiro de 2012 [13].

In a comparison of international standards, grades, limits, and tests for several countries were identified. In many cases, olive oil might be accepted in some countries as EVOO but rejected in others. This lack of uniformity creates trade barriers and is a further complication in fighting fraud.

Detection of Adulteration of EVOO using FTIR Data Analysis

Recent developments in Fourier transform infrared (FT-IR) spectroscopy instrumentation extend the application of this technique to the field of food research, facilitating particularly the studies on edible oils and fats. In this work, FT-IR spectroscopy is used as an effective analytical tool in order to determine extra virgin olive oil adulteration with sunflower, corn and refined olive oils in their binary admixtures in different concentrations (0, 5, 10, 20, 30, 40, 50, 100% - w/w). The spectral region (1300-1000 cm^{-1}) which contains the IR fingerprints of these vegetable oils was found to be very useful in detecting olive oil adulteration. A band shift observed at 3009 cm^{-1} assigned to the =C-H stretching vibration of the cis- double bond, allows the determination of extra virgin olive oil adulteration. The intensities of the spectral bands at 1163 cm^{-1} (assigned to -C-O stretching vibration and CH₂ bending vibration) increase with increasing adulterant concentration. The absorbance ratio (R_{1118/1097 cm^{-1}}) decreased with increasing adulterant concentration. Also, there was a pronounced shift of the peak at 912.78 cm^{-1} (assigned to -HC=CH- cis- double bond, bending out of plane) for pure olive oil to higher wave numbers with increasing adulterant concentration [14].

Figures 1-4 display these spectral fingerprints in which one can visualize easily that the intensity of the absorption peak at 1163 cm^{-1} decreases by increasing the concentration of sunflower, corn, and refined olive oils, respectively.

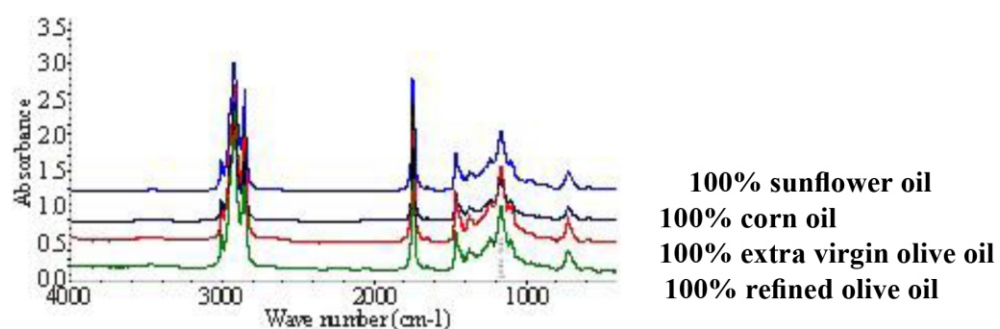


Figure 1: Typical FTIR Absorption spectra (4000-400 cm^{-1}) of pure oils (sunflower, corn, extra virgin olive and refined olive oils)

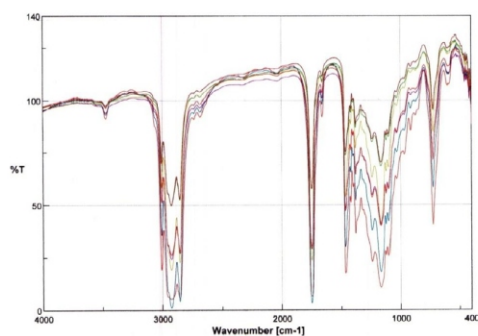


Figure 2: Typical FTIR spectra of pure olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of sunflower oil (bottom).

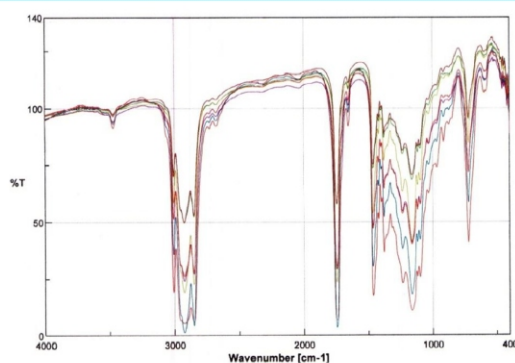


Figure 3: Typical FTIR spectra of pure olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of corn oil (bottom).

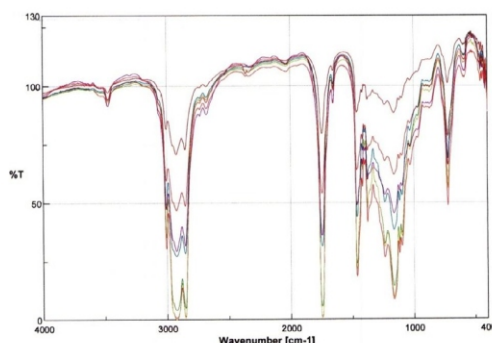


Figure 4: Typical FTIR spectra of pure olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of refined olive oil (bottom).

Detection of Adulteration of EVOO using UV Spectroscopy

Spectroscopic examination in the ultraviolet (UV) can provide information on the quality of an oil, its state of preservation and changes brought about by technological processes. The absorption at the wavelengths 232 nm and 270 nm in cyclohexane is due to the presence of conjugated diene and triene systems, respectively, resulting from oxidation processes and/or refining practices. These absorptions are expressed as specific extinctions conventionally indicated by K (also referred to as “extinction coefficient”). In this work, the specific extinctions at 232 nm and 270 nm in cyclohexane are calculated for a concentration of 1% w/v for extra virgin olive oil adulteration with sunflower, corn oils in their binary admixtures in different concentrations (0, 5, 15, 25 and 100% w/w). According to the regulations and recommendations stated in the official Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive- residue oil and on the relevant methods of analysis [6], the extinction coefficients K₂₃₂ and K₂₇₀ for extra virgin olive oil (EVOO) should be ≤ 2.5 and ≤ 0.22 , respectively and ΔK should be ≤ 0.01 . Results revealed that the adulteration will be detected easily by UV absorbance at 270 nm because sunflower oil showed a shift out of the permitted range at 15% upward higher concentrations added to extra virgin olive oil. Also, corn oil will be detected if present in 15% upward higher concentrations in blends with extra virgin olive oil. The calculated ΔK allowed the detection of adulteration in extra virgin olive oil at the concentration of 15% upward higher concentrations of adulterant sunflower and corn oils; and this is the lowest detectable value in adulterated extra virgin olive oil [15].

Detection of Adulteration of EVOO using Differential Scanning Calorimetry (DSC)

The aim of this research was to identify the added refined olive oil (ROO) to extra virgin olive oil. One sample of each oil was analyzed, admixtures of EVOO: ROO were prepared at different ratios (50:50, 60:40, 70:30, 80:20, 90:10

and 95:5 w/w). To identify this adulteration, exothermic and endothermic thermograms were investigated. The results indicated that by increasing refined olive oil, onset temperature (Ton) was increased from -61.08 to -56.16 °C; also, offset temperature (Toff) decreased from 19.09 to 12.57 °C. Also results showed that when the ROO ratio increased then the enthalpy average values of both exothermic and endothermic curves were declined. This matter is subject to changes in fatty acids, since by adding more refined olive oil, the amount of oleic acid, which is a monounsaturated fatty acid was increased, and the amount of linoleic acid, which is polyunsaturated fatty acid, decreased. Ton relates to melting monounsaturated triacylglycerols and Toff relates to crystallization of polyunsaturated triacylglycerols [16].

Detection of Adulteration of EVOO using UHPLC-CAD profiling of triacylglycerols and PCA

Ultra-high-performance liquid chromatography (UHPLC) with charged aerosol detection (CAD) was employed to characterize EVOO along with potential adulterant oils based on their triacylglycerol (TAG) profiles. Statistical analysis of these TAGs using principal component analysis (PCA) allows for a rapid approach to determine EVOO authenticity. Using this approach, adulteration of EVOO with cheaper vegetable and seed oils and lower-quality olive oils had detection limits at or below 10%, depending on the adulterant. Compared to traditional methods, UHPLC-CAD with PCA involves minimal sample preparation combined with fast analysis, for a rapid determination of EVOO authenticity [17].

CONCLUSIONS

The adulteration of olive oil, particularly EVOO, is prolific because the financial gains are large and the availability of the highest grades low. Seed oils from broad-acre farming are relatively inexpensive due to the lower cost of production and the more efficient (solvent extraction) methods of processing. Lower quality olive oils are more abundant and cheaper to produce. There is a significant financial gain for fraudsters who might blend seed oils or refined/lower quality olive oils and sell them as EVOO. Because testing is sporadic and expensive, the chance of being caught is relatively remote and

the potential consequences not severe under the law of most countries. Blending a small portion of aromatic EVOO with tasteless, colorless, refined seed oil can produce a product with some sensory and visually characteristics that make it appear to be authentic. Adulteration of EVOO with seed oil and/or refined and/or poor quality olive oil is not known to cause severe health issues (with the exception of the toxic oil syndrome cases in 1981). However, the main issue is more likely the decline in health benefits due to degradation of beneficial components of EVOO. Despite this, consumers continue to buy olive oil for the perceived and documented nutritional benefits and for the sensory qualities of freshness and fruitiness. Clearly, a more determined global effort from authorities is required to overcome the corrupt practices. More testing of products, particularly from government facilities, is warranted to ensure the oils meet the expectations expressed on the label. There needs to be more government support in export/import situations and higher penalties for those that abuse the regulations.

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Jojoba oil in the cosmetics industry

Introduction

Jojoba oil is the liquid produced in the seed of the *Simmondsia chinensis* (Jojoba) plant, a shrub, which is native to southern Arizona, southern California, and north-western Mexico. The oil makes up approximately 50% of the jojoba seed by weight.

The terms “jojoba oil” and “jojoba wax” are often used interchangeably because the wax visually appears to be a mobile oil, but as a wax it is composed almost entirely (~ 97%) of mono-esters of long-chain fatty acids and alcohols, accompanied by only a tiny fraction of triglyceride esters. This composition accounts for its extreme shelf-life stability and extraordinary resistance to high temperatures, compared with true vegetable oils.

Approximately 50% of the weight of the seed is a mixture of long-chain liquid esters that is typically extracted by mechanical pressing.

Physical properties

Unrefined jojoba oil appears as a clear, golden liquid at room temperature, with a slightly nutty odour. Refined jojoba oil is colourless and odourless. The melting point of jojoba oil is approximately 10°C and the iodine value is approximately 80.

Jojoba oil is relatively shelf-stable when compared with other vegetable oils mainly because it contains few triglycerides, unlike most other vegetable oils. It has an oxidative stability index of approximately 60, which means that it is more shelf-stable than safflower oil, canola oil, almond oil or squalene, but less than castor oil and coconut oil.

Acid value is the most frequently determined property of jojoba oil. It is typically in the range of

0.2-0.5 (mg of KOH to neutralize acid in 1 gram of sample) and it can be reduced to less than 0.2 with refining methods.

The moisture content is typically less than 300 ppm. It typically contains between 50 and 100 ppm of phosphorus in the form of hydratable and non-hydratable phospholipids. The phospholipids are considered a positive component in many cosmetic applications, but too high a level may cause turbidity and cloudiness in the Jojoba.

The peroxide value is used as a measure of its relative oxidation state at a single point in time. Typically, Jojoba's peroxide value at time of packaging is less than 0.8 milliequivalents of peroxide per kg of sample.

Iodine Value (IV) is a measure of unsaturation (Number of double bonds). Jojoba oil is almost exclusively composed of diene, making its IV similar to that of oleic acid. IVs are typically in the range between 78-90.

The Saponification Number ranges from 85-100.

Chemical Composition

The fatty acid content of Jojoba oil can vary significantly, depending on the soil and climate in which the plant is grown, as well as when it is harvested and how the oil is processed. In general, it contains a high proportion of mono-unsaturated fatty acids, primarily 11-Eicosenoic acid (Gondoic acid).

Stereochemically, Jojoba's double bonds are cis in character. It is believed that this cis configuration in Jojoba's linear ester molecules actually adds superior emolliency traits to the natural jojoba esters.

Fatty acids present in jojoba oil

Fatty acid	Percentage (Mole fraction)
Palmitic acid	0.3
Palmitoleic acid	0.3
Stearic acid	0.2
Oleic acid	9.3
Arachidic acid	-
11-Eicosenoic acid	76.7
Behenic acid	Trace
Erucic acid	12.1
Lignoceric acid	0.1
Nervonic acid	1.0

It has rich presence of skin nourishing nutrients like Vitamin E, copper, selenium, zinc, chromium, silicon, B-complex and iodine.

Unlike most triglyceride seed and nut crude oils, Jojoba oil is very low in or virtually devoid of tars, gums, free carboxylic acids, hydroperoxides, phosphatides, chlorophylls, colour bodies and malodorous low molecular weight carbonyl compounds. Expelled, crude jojoba is naturally of high quality and purity as it flows from the mechanical presses.

Applications

Being derived from a plant that is slow growing and difficult to cultivate, jojoba oil is mainly used for small-scale applications such as pharmaceutical and cosmetics. Overall, it is used as a replacement for whale oil and its derivatives, such as cetyl alcohol. The ban on importing whale oil to the US in 1971 led to the discovery that jojoba oil is in many regards superior to sperm whale oil for applications in the cosmetics and other industries.

Jojoba oil is found as an additive in many cosmetic products, especially those marketed as being made from natural ingredients. Products commonly containing jojoba include lotions and moisturizers, hair shampoos and conditioners.

The pure oil itself may also be used on skin, hair or cuticles.

Skin care

Many of the most effective ingredients for skin care formulations are those with chemical composition and physical properties similar to the skin's own surface layers. Since Jojoba is completely miscible with sebum, it forms a very thin, non-greasy lipoid layer of jojoba oil and sebum when it is applied to the skin. This partially porous layer provides exceptional transepidermal respiration and moisture control. Unlike greasy occlusive materials such as petroleum, mineral oils and some lanolin products, jojoba oil provides an absolutely non-tacky and non-greasy, dry emolliency.

At the same time Jojoba oil significantly reduces Transepidermal Water Loss (TEWL) without totally blocking transpiration of gases and water vapour. From the pores and hair follicles, Jojoba oil diffuses into the corneal layer of the skin probably via a pilosebaceous mechanism.

In short, it appears that Jojoba oil effectively moisturizes and softens the skin by a dual action of forming a lipid layer, which is partially occlusive, and by the diffusion of Jojoba into the inter-cellular spaces of the stratum corneum to soften this tissue.

The incorporation of Jojoba into the oil phase of skin care formulations is a straightforward process. Jojoba oil has a required hydrophilic/lipophilic balance number (HLB) of approximately 6. It is considered compatible with almost all anionic, cationic, amphoteric and non-ionic cosmetic ingredients. Not only can multi-functional Jojoba oil be considered as a replacement for mineral oil, triglycerides, lanolin, squalane and synthetic esters, but it can bring a whole new level of functionality to products.

Jojoba oil aligns with customer demand for clean beauty; it can repair and moisturize irritated and sun-damaged skin. In a study published by R. Habashy in the Pharmacological Research Journal, it was found that Jojoba oil applied on irritated skin exerts anti-inflammatory activity similar to a non-steroidal anti-inflammatory drug (NSAID) used to relieve pain and swelling – which can be especially useful in after-sun products. Jojoba oil is safe to use on all skin types, even on sun-injured skin, assisting the skin in its rejuvenation process.

In a study published by E. Ranzato in the Journal of Ethnopharmacology, it was found that Jojoba oil accelerates wound healing by stimulating collagen synthesis in in-vivo tissues. Many oils can feel heavy and clog skin pores, but Jojoba oil doesn't cause breakouts. In fact, it could actually prevent them! Since it is biocompatible, it absorbs into the skin rather than sitting on top of it.

Jojoba oil also helps in oil control. Since it keeps the skin well-hydrated, the body naturally reacts by decreasing sebum production in the affected area.

Not only is Jojoba oil moisturizing, but it also has antibacterial and immune-boosting properties to help heal wounds. The iodine in Jojoba oil protects the skin from bacteria and the Vitamin E and B-Complex Vitamins help boost the skin's immune response.

There are a handful of natural oils that provide UV protection, and Jojoba oil is one of them. It has a Sun Protection Factor (SPF) of 4, which is great when paired with other SPF ingredients. It has also been found to slow the effect of UV exposure on collagen, meaning it can reduce the ageing effect of the sun.

Hair care

Jojoba oil is an extremely functional ingredient in hair and scalp preparations. Many scalp related

problems are caused by a hardened build-up of sebum that clogs the hair follicles and may cause some types of scaling. If this hardened build-up is not removed, it can eventually obstruct the hair follicle's ability to function properly, which can lead to a loss of hair shaft, and ultimately, death of the follicle. Jojoba oil rapidly penetrates down to the scalp hair shaft, and readily loosens and dissolves the hardened build-up. The scalp and hair follicles are left clean and free to continue their normal function.

Jojoba oil is also an excellent soil-solubilizing agent, which can remove sticky build-up on the hair from many modern hair preparations as well as airborne particulates. Jojoba oil will leave the hair clean and supple. It exhibits a matchless keratoplastic effect that leaves the hair shimmering and brings out the hair's natural colour overtones and brilliance. Jojoba oil can be used with confidence in most hair preparations at a level ranging from 0.5-3%.

Jojoba oil is commonly used and added into shampoos and conditioners to provide extra conditioning for hair.

It's also added to other conventional, over-the-counter products such as sunscreens, lipsticks and lip-gloss.

Market trends

The cosmetic industry plays a key role in the growth of the Jojoba oil market, which is set to reach US\$254.2 mn by 2024, according to a report by Grand View Research, Inc. Cosmetic was the largest segment of the global market, accounting for 70.6% of the overall revenue in 2015. Cosmetics applications of Jojoba oil will witness the fastest volume growth at a CAGR of 5.5% from 2016 to 2024.

Major cosmetic manufacturers are undertaking extensive research and development for innovation of Jojoba oil – based formulations.

Application of Jojoba oil in cosmetics

Hair care	Skin care	Sun care	Make-up products	Miscellaneous
Shampoos, hair conditioners hair oils	Facial moisturizers	After-sun creams	Cream foundations	Scrubs and masks
Scalp treatments	Facial cleansers	Lotions sun protection products	Liquid foundations	Baby care lotions, creams & oils
Wave set lotions	Eye makeup remover	Self-tanning products	Lipsticks	Cuticle and nail care products
Stick pomades	Eye treatments	Tan extending products	Solid foundations	Bath oils
Hair creams, hair tonics, hair sprays	Body moisturizers	Lip balm	Concealers/blemish sticks	Soaps (liquid and bars)
	Hand creams		Eyeshadow/blusher	Foot care products
	Shaving lotions and after-shave creams and oils		Eyeliners	Massage oils

North America's Jojoba oil market was valued at \$58.1-mn in 2015 and will show a significant rise owing to increasing penetration of the oil in various organic skin care products. Asia-Pacific will witness significant revenue growth at a CAGR of 7.1% from 2016 to 2024 on account of rising demand from Japan and other South-east Asian Countries. The growth of the male grooming industry, particularly in emerging economics of the region, is expected to fuel demand over the forecast period.

Jojoba is used extensively Japan, owing to consumer inclination toward natural ingredients. Japanese oil massages use Jojoba oil.

Conclusions

Jojoba is an emerging crop well suited to production in arid regions, including California,

India, Israel, and Australia. It requires careful management of fertilizer, soil pH, and irrigation to obtain maximum yields.

However, the main limitation to the widespread adoption of the crop and the development of sustainable markets for Jojoba oil is the difficulty in obtaining yield that is stable and reliable. This can best be achieved through vegetative propagation of adapted clonal lines produced for each region/country. Alternatively, the production of Jojoba-type waxes for transgenic canola may provide a means of large-scale production and the establishment of new markets.

As with all new crops, the number and importance of pests of the crop are not well documented, but the crop does appear to have relatively few pests that threaten its production in any country.

Coffee in cosmetics

Coffee is an indispensable part of our lives. Our day starts with a cup of coffee. One might rely on a morning cup of coffee to boost energy and metabolism on a daily basis. While it is scientifically proven that this 'wonder drink', when consumed in moderation, promotes good health and contains properties that help you keep illness at bay, coffee lovers now have one more reason to rejoice as we decipher its numerous benefits for the skin and hair!

Coffea is the genus of flowering plants in the family Rubiaceae. Coffea species are shrubs or small trees native to tropical and southern Africa and tropical Asia. The seeds of some species, called coffee beans, are used to flavour various beverages and products. The fruits, like the seeds, contain a large amount of caffeine, and have a distinct sweet taste and are often juiced. The plant ranks as one of the world's most valuable and widely traded commodity crops and is an important export product of several countries, including those in Central and South America, the Caribbean and Africa.

Coffee supply

There are over 120 species of Coffea, which is grown from seed. The two most popular are Coffea arabica (commonly known simply as Arabica), which accounts for 60 to 80% of the world's coffee production, and Coffea canephora (known as Robusta), which accounts for about 20-40%. C. arabica is preferred for its sweeter taste, while C. canephora has a higher caffeine content. C. arabica has its origins in the highlands of Ethiopia and the Boma Plateau of Sudan, and was the result of a hybrid between C. canephora and C. eugenoides.

The South Indian states are the major producer of coffees in India-especially Karnataka (53%),

Kerala (28%), and Tamil Nadu (11%). Some coffee is also grown Andhra Pradesh, Orissa, Assam and Tripura. Hilly area and good monsoon in these regions make them well placed for coffee and tea. Indian coffee is considered as one of the finest in the world, and grown under the shade rather than under dark sunlight.

Use in personal care

Coffee is a source of nutrients and anti-oxidants that may benefit the skin, scalp and hair. The environment is full free radicals (population particles, etc.) that wreak havoc on the skin. However, loading with the antioxidants in coffee protects it and bolsters its natural defence.

Infact, the American Chemical Society has found that coffee is the most popular source of antioxidants in the United States – even more so than other antioxidant-rich beverages, such as tea and wine. A person can use it to exfoliate, treat acne, increase blood flow, and balance pH levels.

Use in skin care

Exfoliation

Coffee grounds make a great exfoliant. The grounds do not dissolve in water, which makes them good at scrubbing away dead skin cells. Results of a study from 2013 suggest that substances in coffee also help to promote healthy skin. Caffeic acid, an antioxidant, may boost collagen levels and reduce the premature aging of cells. Caffeic acid also has antimicrobial properties, which means that it may help protect the skin against germs. Confirming these findings will require more studies in humans.

Getting rid of puffy eyes

Coffee may benefit people with puffy, inflamed eyes. Caffeine stimulates blood flow and widens, or dilates the blood vessels. This increases blood flow, which can help the skin to naturally tighten.

The result may be a reduction in the buildup of fluid under the eyes.

Other compounds in coffee, such as Chlorogenic acids, may also reduce inflammation around the eyes.

Sun protection

Coffee contains antioxidants, such as polyphenols, that may help to protect against ultraviolet (UV) rays, and some signs of aging linked to sun exposure. Authors of a study from 2015 concluded that participants with the highest consumption of polyphenols, from coffee or other sources, had fewer UV age spots on their faces.

Cellulite reduction

Coffee may also help reduce the appearance cellulite on the skin. A small study reported that a topical slimming product containing caffeine and several other active ingredients was more effective at reducing stubborn cellulite in women than a placebo product.

Acne treatment

The antioxidants, stimulants, and chlorogenic acids in coffee may make it an effective acne-fighting facial scrub.

Acne occurs when oil, dead skin cells, and other substances clog pores, which can become infected by bacteria, resulting in inflammation.

Foot bath

Coffee may be very effective at cleansing the feet and smoothing and softening the skin. The grounds can help scrape away dead skin cells from the soles of the feet, and the stimulating effects if caffeine may help to increase blood flow and circulation in the area.

Use for hair and scalp care

Rubbing coffee grounds into the scalp can scrub away dead skin cells. The scalp and hair are naturally acidic. Coffee is naturally acidic. The American Dental Association gives a pH value of

5.11 to the medium roast of a major brand. Applying coffee to the hair can be a great way to help rebalance the pH levels of the hair and scalp.

Coffee is known to boost hair growth. It is caffeine that helps to stimulate the hair follicles to promote hair growth. Here's how. Dihydrotestosterone (DHT) is a major component in determining hair growth. DHT when broken down by certain enzymes helps promote hair growth. However, when these enzymes failed to break it down, DHT starts to buildup and that weakens the hair follicles and tampers with the integrity of your hair, thereby stopping hair growth. That is where caffeine comes in. Researchers have found that caffeine helps to block the build-up of DHT, improving the blood circulation in the scalp, and stimulating the hair follicles to boost hair growth. Regular use of coffee on the hair strengthens the hair follicles and makes hair soft, smooth and long.

According to the American National Centre for Biotechnology Information (NCBI), coffee directly activates cells in hair follicles by releasing ATP. ATP is a molecule that carries energies between cells. It can also combat DHT, thus reducing hair fall and making your hair stronger. Coffee not only makes hair stronger, but it also improves its quality and makes them soft and shiny. Wondering how? Well, coffee contains flavonoids that fight dullness and dryness.

It boosts blood circulation to the scalp. When applied to the scalp, coffee improves blood circulation. This helps to transfer nutrients to the hair roots. As a result, hair grows faster and becomes thicker.

Coffee is great for detoxifying the scalp. It allowing you deal with flakiness and dandruff while also balancing the pH level.

End-notes

Coffee is multi-purpose ingredient that can potentially offer a variety of skin, scalp and hair care benefits.

OBITUARY

Write up of Shri Agam Swaroop Khanna (A. S. Khanna)



Dr. A. S. Khanna, one of the founder members of OTAI (E.Z.) A LIFE TIME ACHIEVEMENT AWARDEE left for heavenly abode on the 18th June 2021 in N. Delhi. Born on 4th March 1925 in zamindar family, in Bijnor (UP), a very disciplined child with zeal to learn made him most studious among his six siblings. He passed his matriculation from Moradabad as during those days, English medium school closest to Bijnor was at Muradabad. He then came to Kanpur to study B.Sc. from DAV college and after that did a Post Graduation course in Oil and Paint technology from HBTI, Kanpur. While in DAV Hostel, he met his senior, Mr. Atal Behari Vajpayee

who was then a socialite. He joined RSS at young age of 15 years and subsequently Bhartiya Jansangh Party at a later stage of his life. He was a core RSS man. On his RSS guru Mr. Hedgeokar's persuasion, he debated philosophy of RSS and Jansangh with Atalji and was successful in making him join RSS. So in a way credit goes to him, which nobody knows, that he was the one who brought Atalji in RSS and Jansangh party.

He was a very active member holding high flagship positions in Seva bharti, Yogakshem, Vivekanand Society and did of plenty of social work in different fields. He was also a member and president of Rotary club in Behala, and did social work for the blind in Behala blind school. he was a health freak and sports lover, who won many trophies in badminton and table tennis for his club (Hindustan club in Moidan).

He was very actively involved in 70s with Punjabi Beradree and Punjab Club holding different post in which most important was : he was the editor of the punjabi beradree monthly magazine.

All his life he worked for paint industry. He joined Shalimar Paints in 1948, the ICI Paints and Macfarlane Paints in 1969. He was president of All India Paint Association in 1979-80. He started his own paint venture in 1983 and was involved till his end came. He wrote a book on oil and paints at an age of 31 which was a textbook of paints in HBTI.

He was one of the founder members of OTA and was involved in all major activities of the association throughout his Life and was the first Editor of E.Z. News Letter.

A very health conscious person was very focused on nutritive diet (except sweets especially his love for sandesh) which drove him to right a book on 'Food and Nutrition' but unfortunately his death left it incomplete.

Very few know that he was also a qualified homeopath.

O.T.A.I. members express their heartfelt condolences at this hour of bereavement and pray for His Soul to rest in Peace!!

PARLIAMENT NEWS

Direct Procurement of Farmers' Produce

Rajya Sabha Starred Question No. 187 Answered on 12th March 2021

*187, Smt. Jharna Das Baidya

Will the Minister of Agriculture And Farmers Welfare be pleased to state :

- (a) whether any concrete measure is being initiated for direct procurement of farmers' produce;
- (b) if so, the details thereof, State-wise; and
- (c) if not, the reasons therefor?

ANSWER

Minister Of Agriculture And Farmers Welfare (Shri Narendra Singh Tomar)

(a) to (c): A statement is laid on the Table of the House.

Statement In Respect Of Parts (a) to (c) of the Rajya Sabha Starred Question No. 187

(a) to (c) : The procurement of farmers produce as part of Minimum Support Price (MSP) operations is done from farmers directly in all the states except in Punjab and Haryana.

The Central Government extends price support to wheat and paddy through FCI and State Agencies. Under this scheme, registration of the farmers are done through online portal and whatever food grains are offered by such registered farmers, within the stipulated procurement period and which conforms to the prescribed quality specifications, are purchased at MSP by the Government agencies including FCI. The Government agencies undertake MSP operation at mandis/temporary purchase centres/aggregation points. Location and number of purchase centres to be opened are decided in consultation with / by the State Governments. The procurement of paddy and wheat are done through centralized and decentralized procurement system. Under centralized procurement system, the procurement of foodgrains in Central Pool is undertaken either by FCI directly or by State Govt. Agencies (SGA). Under decentralized procurement system, the State Government/its agencies procure rice / wheat / coarse grains within the state. The excess stocks (Rice & Wheat) procured by the State /its agencies are handed over to FCI in Central Pool. The Coarse grains are procured by the State Governments based on their plan of procurement with prior approval of GOI from the registered farmers.

The Procurement of MSP notified pulses, oilseed and copra are done under Price Support Scheme (PSS) based on the request of the concerned State Government. The procurement under PSS is done by central nodal agencies through state designated procuring agencies directly from the farmers

registered in online portal. All the payment of the MSP value are done through online mode to the registered farmers. The procurement centres are opened by the Central Nodal Agencies in consultation with the State Government considering the convenience of the farmers.

The Cotton Corporation of India (CCI) has been appointed as the central nodal agency for undertaking price support operations in the event, prices of Fair Average Quality (FAQ) grade kapas fall below the MSP level. These procurement operations are carried out only in Agricultural Produce Market Committee (APMC) yards directly from the farmers duly identified on the basis of their land records. AADHAR cards, bank details etc. without involvement of any middlemen and payment to cotton farmers are made 100% through online directly in farmers' bank account after receipt of purchase bill along with details of farmers from APMCs.

For Jute, Jute Corporation of India (JCI) is conducting MSP operation as and when required. Raw Jute is transacted in over 500 centres countrywide. The farmers bring their produce to the nearest departmental purchase centre of JCI. The payment for such procured goods is released by direct bank transfer into the bank account of the farmer.

Besides, instruction has been issued by the government for mandatory use of Expenditure Advance Transfer (EAT) module of Public Finance Management System (PFMS) wherein all the payments is to be made through online directly into farmers banks account. This instruction are being followed by all the states except Punjab wherein MSP payment are being paid through arthiyas (commission agent). Recently, the Government of India vide its letter dated 04.03.2021 has directed Government of Punjab to implement guidelines of Ministry of finance regarding compliance of Public Financial Management System (PFMS) and implementation of online full MSP payment to farmers' bank account directly with effect from the upcoming Rabi Marketing Season (RMS) 2021-22.

In Haryana, for procurement of foodgrains made by Food Corporation of India (FCI), payment of MSP is being made directly to farmer's bank account through online mode. However, for the procurement made by State agencies, the payment of MSP to the farmers is made partially through arthiyas and partially directly to farmer's accounts through online mode as per options given by farmers in e-kharid portal. The Government of India vide its letter dated 04.03.2021 has directed Government of Haryana also to implement guidelines of Ministry of Finance regarding compliance of Public Financial Management System (PFMS) and implementation of online full MSP payment to farmers' bank account directly with effect from the upcoming Rabi Marketing Season (RMS) 2021-22.

Prof. D. K. Bhattacharyya

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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

21-06-2003

Regarding availability/price enquiries may be made to :
S. K. ROY, President OTAI (EZ)
5C, Tarak Mitra Lane, Kolkata - 700 026
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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 bioconversion 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



Dr. S. Bandopadhyaya
President



Mr. B. P. Manchanda
Hon. Secretary

QUALITY PHILOSOPHY

- ☐ Quality means clearly identifying customer needs both internal and external and meeting these requirements without error first time every time.
- ☐ Quality is comprehensive and applies to all business activities.
- ☐ Quality is achieved only by the commitment of every individual in the company. Appropriate skills and attitudes are essential elements of this commitment.
- ☐ Quality is built into the process; It comes through prevention rather than inspection.
- ☐ Quality is measurable by the cost of non-conformance to requirements.
- ☐ A spirit of partnership with the supplier and other business associates plays an integral part in the quality improvement process.
- ☐ Quality improvement is a continuous process.

B. P. Manchanda



S. K. Roy with Prof Dr. Augustine Ong soon, Father of Malaysian Palm Oil, President M. O. S. T. A., in Oct 2016 Prof. ONG is well known to Prof. D. K. Bhattacharyya. Our Gov't has recently earmarked Rs. 11,000/- crore for the development of Palm Oil to bridge the Gap between Demand & Supply of Veg Oils. Malaysia is one of the Prime exporters of Palm Oil into India,

Prime Minister announces Rs. 11,000-crore palm oil mission

Prime Minister Narendra Modi has announced a national initiative on palm oil production to help increase farm incomes. The scheme, called National Edible Oil Mission Oil Palm (NMEO-OP), for self-reliance in edible oil involves investment of over Rs. 11,000 crore.

The Centre plans to raise the domestic production of palm oil by three times to 1.1-mt by 2025-26. This will involve raising the area under oil palm cultivation to 1.0 mn hectares by 2025-26 and 1.67 mn hectares by 2029-30. The special emphasis of the scheme will be in India's north eastern states and the Andaman & Nicobar Islands due to the conducive weather conditions in the regions. Under the scheme, oil palm farmers will be provided financial assistance and will get remuneration under a price and viability formula.

Palm oil is currently the world's most consumed vegetable oil. Top consumers of the commodity are India, China, and the European Union (EU). Palm oil is also used extensively in the production of detergents; plastics, cosmetics, and biofuels.

India is the largest consumer of vegetable oils in the world. In 2016-17, the total domestic consumption of palm oil by India was 9.3 mt, with 98.97% of it imported from Malaysia and Indonesia.

Interestingly, the EU and China use only 46% and 58% respectively of their palm oil in food-related products, while the rest goes into cosmetics, oleochemical, and pharmaceutical products. In India, 94% of palm oil is used in food products, especially for cooking purpose. This makes palm oil extremely critical to India's edible oils economy.

Fatty acids composition of some edible oils. Percentage by weight.

Fat / Oil	SAFA	MUFA Oleic	PUFA		
			Linoleic	Linolenic	Total
Coconut Oil	87.9	7.8	0.8	----	0.8
Palm Oil	47.9	37.9	9.0	---	9.0
Musta / rapseed	10.7	56.7	18.1	14.5	32.6
Safflower Oil	10.7	17.7	78.5	----	78.5
Soybean Oil	13.1	28.9	50.7	6.5	57.2
Sesame Oil	13.4	41.3	45.3	---	45.3
Sunflower Oil	9.1	25.1	66.2	----	66.2
Cottonseed Oil	25.9	22.9	47.8	----	47.8
Groundnut Oil	20.9	49.3	29.8	----	29.8
Rice Bran Oil	18.0	45.0	35.0	2.0	37.0
Olive Oil	9.0	75.0	16.0		

The edible fats and oils shown above are only divided into groups on the basis of their contents of different classes of fatty acids.

1. Fats rich in SAFA

AHA - SAFA:MUFA:PUFA = 1:1:1
2. Fats rich in MUFA

Japan Min of Health
3. Fats highly rich in PUFA
4. Fats rich in PUFA

SAFA:MUFA:PUFA = 1:1.5:1
5. Fats more or less balanced