Biochemical Analysis of Organic and Conventional Coffee Seeds (*Coffea arabica* L.): A comparison

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Abstract – **Chemical components in coffee seeds (*Coffea arabica* L.) from conventional and organic fields were analysed by Gas Chromatography-Mass Spectrometry (GC-MS) for comparison. Visual analysis of the chromatograms of two different coffee seed extracts showed that there were some differences between their chromatogram profiles despite number of peaks obtained for both extracts were same. The GC-MS analysis of coffee seeds from chemical field showed 12 different peaks. Peak no.1 at RT 21.954 was matched with three compounds including Caffeine and anthrone. Totally 9 compounds in the extract showed exact match with the library since their quality was more than 90%. Among the different compounds, Caffeine (25.07%) and n-Hexadecanoic acid (25.17%) were found to be the major compounds in the extract. The coffee seed extract from organic field also showed 12 peaks and 6 compounds were confirmed as their quality exceeded 90%. Health benefits and hazards of both chemically and organically grown coffee were also studied from the results of the biochemical analysis.**

Key words – **Arabica coffee beans, chemical components, conventional coffee, GC-MS, natural coffee**

I. INTRODUCTION

Coffee beans are the seeds of a perennial evergreen tropical plant, which belongs to the family Rubiceae and genusCoffea. Coffee is a popular beverage that is obtained from different varieties of coffee beans.A healthy coffee is the first taste to begin the day with. The people who enjoy a cup of coffee in the morning prefer and expect a pure and healthy drink to begin the day. For many coffee lovers coffee is considered as the energizer and a mind activating agent. This important drink that contains many micronutrients, some of which have proven bioactivities, Such as anticancer, antimicrobial, antioxidant, and host of other effects. Hence a good and perfect coffee is an essential requirement of the market. The distinct flavor of brewed coffee is certainly the main reason for its wide popularity and almost universalappeal as a refreshing beverage [1]. Coffeaarabica dominates the world trade due to its superiorquality.It is the Botanists' duty to produce the right coffee. Thus Botanists can contribute to a great level to the common good. Some important groups of these biologically important micronutrients, like caffeine have been discussed in this research. Because of their importance in biological systems, recent analytical methods for their characterization have also been reviewed. It is envisaged that proper long way in aiding quality assurance and safeguarding consumer safety and satisfaction.

Generally Coffee is the major traded commodity next to oil and thus plays a vital role in the economy of all nations. Coffee is the major non-alcoholic beverage consumed with cheer and preference. Hence coffee guarantees a solid basis for promotion of health and economic development. Moreover, organic coffee is winning the interest of the premium market price across the world thereby increasing the economic return of

coffee producers. Despite the market opportunities and better market price for organic coffee, there are several factors which drastically affect organic coffee production and profitability under small holder farmers. If this can be achieved through research and constant efforts drinking coffee would be a pleasant and wanted experience by all.The "organic" food usually grown with practices that:Do rely on natural biological systems for pest and weed control thus avoiding use of synthetic pesticides, herbicides and soil fumigants.Do improve the quality and fertility of the soil. Do protect water quality. Do reduce soil erosion. Do reduce the impact of agriculture on our environment. Do produce high quality, great tasting food. Don't use genetic engineering. Don't use sewage sludge as fertilizer [2]

In general Organic agriculture is a system of food production and consumption aimed at environment friendly and health-conscious consumers. Organic farming has the potential to provide benefits in terms of environmental protection, conservation of non-renewable resources, improved food quality, reduction in output of surplus products and the reorientation of agriculture towards areas of market demand [3].

II. PRODUCTION OF COFFEE

A. What could be the Healthiest Coffee in the World?

Looks good, Smells good, Tastes good...but does it haveresidual pesticides?

Socially responsible coffee, “Counter Culture Coffee”

An Organic coffee is a revolutionary coffee that can repair the damage done to our body by harmful inorganic substances in a coffee consumed on a daily basis. Drinking regular coffee (Chemical fertilizer used) is equivalent to drinking acid and addictive just like drugs. Organic Coffee, the only alkaline coffee, can give us the ability to drink coffee without all the negative side effects. We can receive a natural energy boost without a crash, with the added bonus of supplementing nutrition we miss out from the foods we eat daily. In the recent past the dominance of the use of chemical fertilizers and chemical pesticides, fungicides and herbicides has spoiled and polluted the land and water and the produced coffee to a great extent. This has done a great damage to the human and nature’s health to the maximum. Hence it is the need of the hour to change our hearts and minds to save the environment of our green earth from the hazards of chemicals, because coffee cultivation very much can help in protecting our green hills from soil erosion and richness of the soil and its development. Ideally, organic farming views the farm as an ecosystem unto itself—one that must be kept healthy and in a state of balance. After tobacco and cotton, conventionally-produced coffee is the third most heavily chemically treated crop in the world. Not only are some of the synthetic pesticides and fertilizers used banned in most western nations; they’re often used without any genuine regulatory supervision. Organic coffee farms as havens for biodiversity and “shade-tree” or “bird-friendly’’ coffee as a new conservation-oriented coffee farms.

B. Indian Scenario:

India is the 6th largest coffee producer in the world. It is successfully grown in the southern states of Karnataka (70 %), Kerala (20%), and Tamil Nadu (7%), specifically in the plantation districts within the Nilgiris Bio-sphere that are known to be ecologically sensitive region of the country. Small growers (with less than 10 hectares) contribute substantially in coffee sector, in terms of no of holdings and production. They account for

90% of operational holdings and 70% of the production.Hence it is major source of income for the small andmarginal planters as well as plantation workers. As a production system,it is labour intensive. Farm labour in coffee plantations requires a particular set of skills and in the light of limited options for mechanization, coffee production is basically recognized as labour intensive and hence it is exposed to a high level of farm risk (production and market risk) [4]

C. **Sirumalai Coffee**

Loyola Estate, Sirumalai, Dindigul District of Tamil Nadu is the area from where the organic coffee produced and taken for research. Arabica coffee is mainly grown in Sirumalai highlands above 800m-1600m ASL. It is wetprocessed to give high quality bean. The recommended Catimor cultivar is taken for research as it is rust resistant. Growing Arabica coffee provides cash income for Sirumalaihillside farmersandreduces the problem of traditional slash-and-burn shifting agriculture. Mostly shaded andcoffees are grown. With the policy of natural resource conservation and thelimitation of land area, theSirumalaihillsidefarmersare growing coffee to sustain naturalresources on the highlands in the long term.

Agro-forestry systems have been introduced for arabica coffee. These systems involve coffee inter-planted with fruit trees and/or forest trees which can provide appropriate additional income to the farmers.(Pepper, Lemon, Orange, pears, Jackfruit tree, and timber trees,) Limiting the use of chemical inputs, including fertilizers and pesticides, is aimed at reducing water and soil contamination and improved ecological conditions as well as the health of the farmers. Organic coffee cultivation on the highlands meets with such objectives and is being encouraged.

Organic coffee cultivation involves: (i) no use of chemical fertilizers, pesticides, herbicides, fungicides, hormones, antibiotic, or growth regulators;(minimum of three years) (ii) use of compost, farm manure, green manure, and crop rotation to maintain and improve soil fertility;(iii) a balanced pest control farm eco-system, with healthy soil management and crop diversification; (iv) control of weeds through mechanical methods; and (v) use of good quality, clean, uncontaminated chemical-free composted materials and nursery seedlings from off-farm as well as on-farm. With organic coffee production, the farm has to be visited and re-evaluated annually, before certification is given. Depending on market demand for organic coffee, there is a high potential for its production on theSirumalai highlands, and organic production methods will be of mutual benefit to the farmers, the highland ecology, and the consumers. Organic coffee-growing areas were special areas which can be strictly controlled and which follow the regulations.

C. Effects of Organic Coffee on the Environment:

Organic agriculture can strengthen the natural environment's resistance to disease. For example, coffee of this standard is generally shade-grown, a quality that promotes forest preservation. Other benefits of this process include the minimization of soil erosion and participation in a healthy ecosystem. Bird populations develop mutually beneficial relationships with coffee fields, enjoying the habitat while keeping insect populations under control and naturally fertilizing the soil.

D. Organic Fertilizers and their Value on Coffee

Great coffee begins with great soil health. Organic manures are important sources of coffee nutrients. This maintains and restores active soil life and health[5]. Organic/Bio fertilizers apparently environment and farmers friendly renewable source of non-bulky, low cost organic agro- input[6]. Organic fertilizers are a huge factor in dictating whether coffee can be certified organic. Organic fertilizers can reduce soil erosion and increase fertility by lowering bulk density [7].This means that farmers are not only growing healthy coffee, but they are putting vital nutrients back into their soils to help the next crop. The coffee plant has a vital nutrient it produces — coffee pulp. Coffee pulp is the outside of the plant that can be salvaged and returned to the soil as an organic fertilizer. Nitrogen, phosphorus, and potassium are the major nutrients that coffee plants need so by using the coffee pulp, cattle manure, bocachi and compost, and chicken manure and bio-green, farmers are able to supply those essential nutrients to the plant cheaper[8].

Due to organic coffee's higher value (it has the greatest value of any organic import in North America) than that of conventional coffee, it only takes up approximately 3% in volume of North America's coffee market, yet its piece of the market concerning value is slightly greater than that of conventional coffee. Organic coffee accounts for about one-third of all U.S. organic beverage sales [9].

The pest, disease and weed are managed judiciously through proper shade regulations, by adopting integrated management practices of physical, mechanical and biological method. For controlling leaf rust and black rot in coffee Bordeaux mixture can be sprayed as per the recommendations[10].

For controlling weeds, in new clearings, cultural practices such as cover digging 30 cm during second year followed by scuffing 15cm for next 2-3 years during post monsoon (October- November) should be followed. Cultivation of green manure crops/cover crops/grain legume crops, mulching and weed slashing through matchet would help to control the weeds.[11].

Keeping all the norms for Organic coffee cultivation as important the arabica coffee was cultivated and it was taken for the test to find its purity and nutrient value. The Biochemical analysis was done with organically grown coffee and conventionally (routine chemicals used) grown coffee.

For conventional coffee cultivation the chemicals used thrice a year are:

Pesticides: Contact (fungicide), Chloripyriphos 20Ec (Pesticide). Foliar spray

Weedicides: On regular weed control- round up.

Fertilizers: Urea, Rock/super Phosphate, Mouriate of Potash, Neem cake powder. Soil application

Micro Nutrients: Multiplex (Coffee special).Foliar spray.

For Organic Coffee cultivation the organic materials used thrice a year are:

PestManagement: Ponneem, green chilli crush, and Shade maintenance, birds friendly ecosystem, Bivariabasianaand effective micro-organisms.

Weed Management: Mechanical and manual weeding.

Nutrients Management: coffee pulp, cattle manure, compost, chicken manure, bio-green,

Micro nutrients: Panchakavia, Fish Amino Acid and naturalleaf humic acid.

E. General Outcome:

The results in harvest showed a lower yield in organic coffee by three times less than the conventional coffee. The expenditures and labour were higher to organic farming by two times than the conventional coffee. The price in the elite market is seven times higher for Organic coffee than the conventional coffee. The end result proves that organic coffee is a preferred commodity in the local as well as the international market for its beneficial advantages..

III. MATERIALS AND METHODS

One Kilogram of Sun dried 14% coffee seeds from both conventional and organic coffee were taken for the biochemical analysis.

A. Preparation of coffee seed extract:

The kernels of coffee seeds collected from organic and chemical fields were separately ground into coarse powder. These two types of coffee seed powders (100 gm) were soaked in 500ml chloroform separately. After 48 h, the extract was filtered through Whatman no.1 filter paper and the solvent in the extract was evaporated by rotary vacuum evaporator under reduced pressure. The crude extract was used for analysis.

B. Gas Chromatography-Mass Spectrometry (GC-MS) analysis:

Comprehensive two dimensional gas chromatography coupled with time –of-flight mass spectrometry (GC x GC-TOF MS)is ideal for the analysis of such complex samples, because the enhanced separation capacity allows analysts to screen the entire composition in a single analysis, with confident identification of compounds that would ordinarily co-elute[12]

GC-MS analysis was performed with Agilent-Model: 7890 A, MS 5975 with helium as a carrier gas with a linear velocity flow on a J&W 5 Ms column (30m x 0.25 mm ID x 0.25 Film Thickness). Column flow rate was 1.0 ml per min @ constant Flow. The oven programme is given below:

	Rate/deg	**temp ^{0}C**	**Hold (min)**
Step 1		50	3.0
Step 2	10	100	3.0
Step 3	10	300	4.0 (35 min)

The Oven Programme

Injector and detector temperatures were 250^{o}C. The identification of the constituents was performed by computer library search and retention indices. Mass spectra were scanned from mass range of 35 to 800, generating 5.27 scan/s. The individual peaks were identified by retention times and the retention index, compared with those of corresponding reference standards and using the NIST11 library (NIST, Gaithersburg, MD). Percentage compositions of coffee seed extracts were calculated according to the area of the chromatographic peaks.

From the library search, the best match of compound corresponding to a particular peak was considered if the quality of the compound was above 90%

IV. RESULTS AND DISCUSSION

A. GC-MS Analysis

Almost 1000 compounds have been identified in roast coffee extracts with chemical composition varying due to a number of factors, such as coffee bean origin and degree of roasting [13].

Visual analysis of the chromatograms of two different coffee seeds showed that there are some differences between their chromatogram profiles despite number of peaks obtained for both extracts were same.

The GC-MS analysis of coffee seeds from chemical field showed 12 different peaks. Peak no. 1 at RT 21.954 was matched with three compounds including Caffeine and anthrone. Totally 9 compounds in the extract showed exact match with the library since their quality was more than 90% (Table 1).

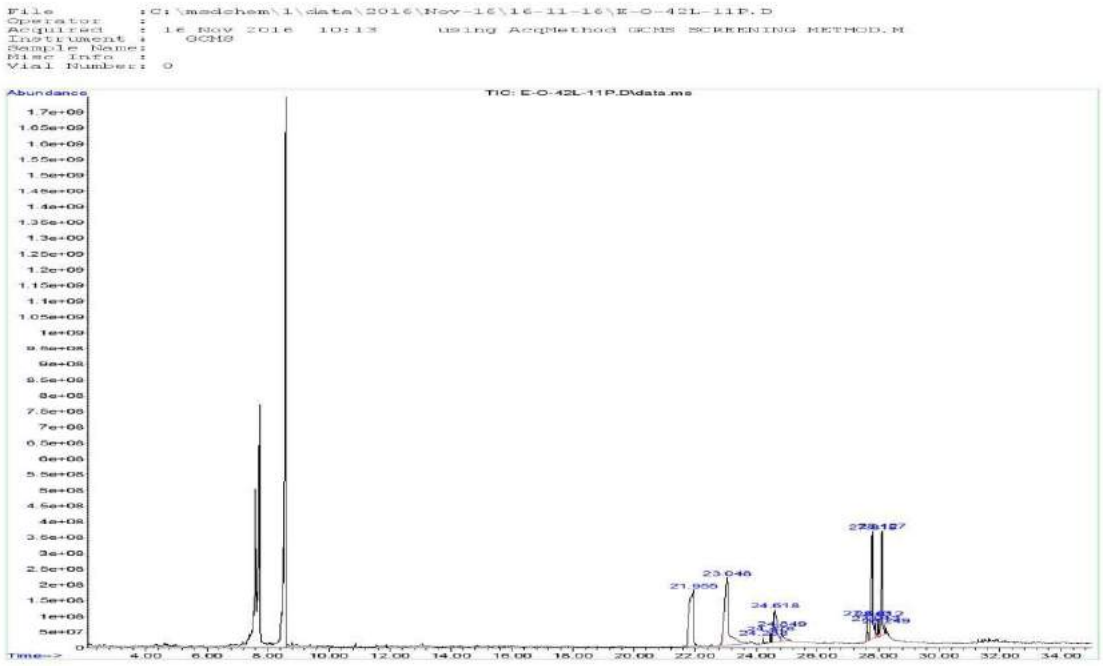
TABLE 1.

COMPOUNDS DETECTED IN EXTRACTS OF COFFEE SEED FROM CHEMICAL FIELD

Sl. No.	Peak Number	RT	Area (%)	Matching compounds
1	2	23.048	25.17	n-Hexadecanoic acid
2	3	24.236	0.49	11-Octadecenoic acid, methyl ester
3	3	24.236	0.49	8-Octadecenoic acid, methyl ester, (E) -
4	3	24.236	0.49	9-Octadecenoic acid (Z)-, methyl ester
5	4	24.476	0.68	Methyl stearate
6	4	24.476	0.68	Heptadecanoic acid, 16-methyl-methyl ester
7	5	24.621	9.47	9,12-Octadecadienoic acid (Z,Z)-
8	5	24.621	9.47	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)
9	6	24.852	2.72	Octadecanoic acid

FIGURE: 1

GAS CHROMATOGRAPHY-MASS SPECTRUM ANALYSIS OF CONVENTIONAL COFFEE SEEDS



Among the different compounds, Caffeine (25.07%) and n-Hexadecanoic acid (25.17%) were found to be the major compounds in the extract.

The coffee seed extract from organic field also showed 12 peaks and 6 compounds are confirmed as their quality exceeded 90% (Table 2).

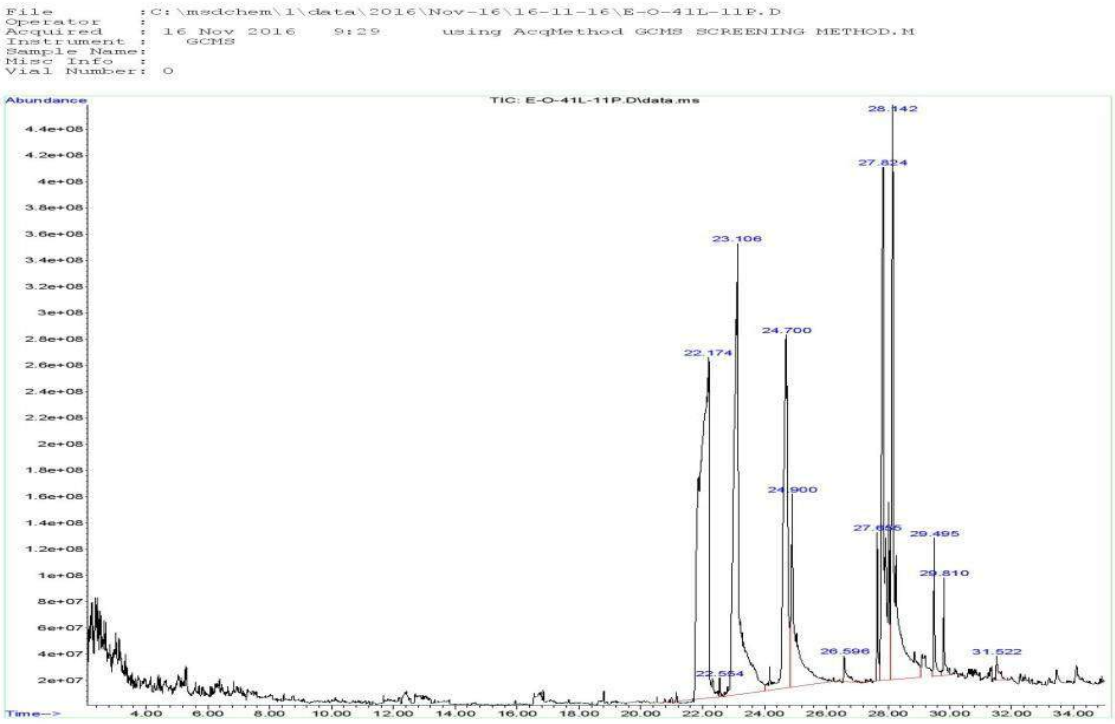
TABLE: 2

COMPOUNDS DETECTED IN EXTRACTS OF COFFEE SEED FROM ORGANIC FIELD

Sl. No.	Peak number	RT	Area (%)	Matching compounds
1	2	22.552	0.19	Pentadecanoic acid, 14-methyl-methyl ester
2	2	22.552	0.19	Hexadecanoic acid, methyl ester
3	5	24.903	7.19	Octadecanoic acid
4	6	26.596	0.61	Octadecanoic acid
5	6	26.596	0.61	Eicosanoic acid
6	12	31.520	0.52	.gamma.-Tocopherol

FIGURE: 2.

GAS CHROMATOGRAPHY-MASS SPECTRUM ANALYSIS OF ORGANIC COFFEE SEEDS



Methyl stearate was detected in chemical coffee only and gamma Tocopherol was found in organic coffee only. Methyl stearate is OCTADECANOIC ACID, METHYL ESTER and it is found in cloves. Gamma (γ)-Tocopherol is the major form of vitamin E in many plant seeds and in the US diet. Recent studies indicate that γ-tocopherol may be important to human health and that it possesses unique features that distinguish it from α-tocopherol.

Hexadecanoic acid concentration was very high in coffee seeds from chemical field. Hexadecanoic acid is the IUPAC name for Palmitic acid. The chemical formula of Palmitic acid is $CH_3 (CH_2)_{14}COOH$. It is the most common saturated fatty acid found in animals, plants and microorganisms [10]. According to the World Health Organization, evidence is "convincing" that consumption of palmitic acid increases the risk of developing cardiovascular diseases [14].

Octadecanoic acid is the IUPAC name for stearic acid. **Stearic acid** is a saturated fatty acid with an 18-carbon chain. It is a waxy solid and its chemical formula is $C_{17}H_{35}CO_2H$.

B. Merits of Organic Coffee:

- Provides over 150 All-Natural Antioxidants to Fight Free Radicals
- Balances PH Level of Body to 7.4,
- Natural Detoxification takes place.
- Leads to Increased Immune System
- Provides Energy and Vigor
- No Jitters or Caffeine Crash due to the presence of Gamma tocopherol (ie) OCTADECANOIC ACID and Methyl ester.
- Oxygenates the Body
- Comes with 100% Certified Organic.
- Supports blood Circulation
- Supports the Quality of Sleep
- Promotes Health and Longevity [15].

C. Demerits of Regular Coffee:

- Dehydrates
- Very Acidic & Toxic. PH is less than 7.
- Raises Blood Pressure
- Raises Stress Level due to Increased Cortisol
- Coffee Jitters & Caffeine Crash due to Hexadecanoic acid(Methyl stearate)
- Causes Heart Disease
- Leads to High Cholesterol Levels
- Allows Fluid Loss in the Body
- Rheumatoid Arthritis due to heavy presence of chemical compounds

- Damages Blood Vessels
- Causes Insomnia
- Anxiety Symptoms
- Source of Depleted Calcium and Iron Levels in Women.[16].

V. CONCLUSION

The combination of chromatographic and mass spectral data from a single analysis provides us a very powerful insight into the taste and health aspects of the complex chemicals in coffee. Users can quickly identify which compounds are largely responsible for the positive and negative health aspects in the organically or conventionally grown coffee. This research data helps us to make the right choice in enjoying a healthy and responsible coffee.The coffee that was grown organically in Sirumalai highlands fulfils all the requirements needed for the certification. The above research proves that the product meets the requirements of the National/international standards on organic production. If taken for certification it would create a transparency among theproducers, buyers and consumers. Certification will surely facilitate a distinct identity and better health and liking through premiums for organic coffee.

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REFERENCES

[1] Petracco, M. (2001). Beverage preparation: brewing trends for the new millennium. In R. J. Clarke, & O. G.Vitzthum (Eds.), Coffee recent developments .Oxford: Blackwell Science.
[2] Pretty and Hine, 2001. An International Conference On Organic Agriculture And Food Security, May 2007. Reducing Food Poverty with Sustainable Agriculture: A Summary Of New Evidence.Michigan State University.
[3] Lampkin, N.H.L.; and Padel, S. (Eds.). 1994.The Economics of Organic Farming: An International Perspective.CAB International, Oxford, England.
[4] Hartmann M., and Akasha B.M., Emerging Challenges for Farm Labour in the Indian Coffee Sector.*Humboldt-UniversitätzuBerlin, Department ofAgricultural Economics* Division of Development Planning and ProjectManagement, p. 1.
[5] The Complete Technology book on Biofertilizer and Organic Farming. {Eds 2}.2012 Introduction to Biofertilizers.NIIRProject Consultancy Services, Delhi, India.P. 13.
[6] The Complete Technology book on Biofertilizer and Organic Farming. {Eds 2}.2012 Introduction to Biofertilizers.NIIRProject Consultancy Services, Delhi, India.P. 13.
[7] Svotwa, E., R. Baipai, and J. Jiyane."Organic Farming in the Small Holder Farming Sector of Zimbabwe." Journal of Organic Systems 4.1 (2009): 1-14. Print.

[8] Valkila, Joni. Fair Trade Organic Coffee Prouduction in Nicargagua -- Sustainable Development or a Poverty Trap?" Ecological Economics 68.12 (2009): 3018-025. Academic Search Complete.Web. 15 Nov. 2011.
[9] Application Note 533. Comprehensive analysis of coffee bean extracts by GCxGC-TOF MS. Marles international. January 2016.
[10] Deivasikamani.S, Sureshkumar.V.B, (Eds). 2014, Coffee Guide, A manual of Coffee cultivation: Organic Coffee production. Central coffee research institute, Karnataka, India. p.180
[11] Deivasikamani.S, Sureshkumar.V.B, (Eds). 2014, Coffee Guide, A manual of Coffee cultivation: Organic Coffee production. Central coffee research institute, Karnataka, India. p.180
[12]cH.D. Beliotz,W. Grosch and P. Schieberie, Food Chemistry (4th Edition),Springer-veriad, 2009,ch.21, pp.938-969.
[13]Gunstone, F. D., John L. Harwood, and Albert J. Dijkstra. The Lipid Handbook with Cd-Rom. 3rd ed. Boca Raton: CRC Press, 2007. ISBN 0849396883 | ISBN 978-0849396885
[14] WHO, 2003, Diet, Nutrition and the Prevention of Chronic Diseases, WHO Technical Report Series 916, Report of a Joint WHO/FAO Expert Consultation, World Health Organization, Geneva, 2003, p. 88 (Table 10)
(15) KoshyEapen, (Eds,).2016. Beyond Modern Medicine: How alkaline are You?.Vasupals'creation Kerala, India.
[16] KoshyEapen, (Eds,).2016. Beyond Modern Medicine: How alkaline are You?.Vasupals'creation Kerala, India.

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