





RESEARCH ARTICLE

Fatty acid profiling in freeze dried Avocado (*Persea americana*) fruit powder in FAME analysis

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Abstract

The study explores the development and characterization of freeze-dried avocado (*Persea americana*) fruit powder to evaluate its potential as a functional food ingredient and its enhanced nutraceutical value. Freshly harvested avocados were procured from Gudalur, Ooty hills, Nilgiris district, Tamil Nadu, India and processed into a freeze-dried powder. Fatty Acid Methyl Ester (FAME) analysis revealed a total fat content of 44.98 %, with 31.05 % monounsaturated fatty acids (MUFA), primarily oleic acid (71 %) known for its cholesterolowering and heart health benefits. The powder also contained 98.70 % saturated fatty acids, 40.70 % polyunsaturated fatty acids (PUFA), trace amounts of tannins (<0.5 %) and trans fats (<0.05 %). Mineral analysis indicated significant levels of calcium (51.13 mg/100 g), magnesium (87.21 mg/100 g), potassium (1198 mg/100 g), (1.23 mg/100 g) and sodium (5.1 mg/100 g). Colorimetric analysis using the Hunter color scale demonstrated a lightness (L*) of 82.96, a greenish hue (a* = -14.32) and a yellow component (b* = 60.38) with a hue angle of 103.34°. The findings suggest that freeze-dried avocado powder retains valuable nutrients and bioactive compounds, making it a promising candidate for use in functional foods and nutraceuticals.

Keywords: avocado; FAME; freeze drying; fruit powder; plant-based ingredients

Introduction

The influence of climatic conditions on fruit production often results in seasonal surpluses, which is an inherent feature of the industry. Fruits are highly perishable and tend to lose some of their quality during post-harvest handling, storage and transport, limiting their potential for fresh consumption. A significant amount of fruit is also wasted at both the wholesale and household levels due to various factors. As fruit is a valuable food source, increasing its consumption should be encouraged. To achieve this, it is important to develop processes and products that can effectively utilize surplus fruit and minimize post-harvest losses, which currently hinder fresh marketing. Reducing fruit waste presents a societal challenge that requires innovative solutions. One viable approach is to create new processed fruit products that can utilize these surpluses and promote consumption and fruit production. Freeze-drying is one such technique that offers a promising solution. This dehydration method produces high-quality, stable and easy -to-handle products, making full use of the edible parts of the fruit and simplifying transport and storage.

In India, matured fruit is particularly consumed as such and mixed with some sweetening agents. The avocado fruit consumption is influenced with its sensory and nutritional attributes. Recently, there has been increasing interest in avocado fruit consumption due to its nutritional value and health benefits. Avocado (*Persea americana*), indigenous to Central and Southern Mexico, is now cultivated globally and is recognized for its nutraceutical properties (1). Avocados are identified for its rich nutrient contents, such as monounsaturated fatty acids, poly unsaturated fatty acids, phytonutrients (xanthophylls, carotenoids, phenols and phytosterols). The consumption of avocados has been associated with various health benefits, includes anti carcinogenic, antioxidant properties as well as hypoglycemic and hypolidemic effect (2).

The avocado tree, scientifically known as *Persea americana* and belonging to the Lauraceae family, is native to central Mexico (3). Commonly referred to as alligator pears or avocado pears, these exotic plants grow quickly and evergreen houseplants. The fruits are green in color, thick skin with fleshy pulp and pear or egg shaped, but

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some are spherical. When ripe, the flesh has a thick consistency like butter and has a light nutty flavor. Hence, the value of this fruit creates strong belief that this decade will solidify the connection between food and health, bringing it into the mainstream.

Avocado fruits are experienced in noticeable postharvest losses that indicated up to 43 % being lost (4). This situation creates avocado as one of the fruits with the highest loss percentages. Current research is indicating postharvest losses for fruits and vegetables worldwide ranged from 28 % to 55 % of total production (5), these high loss rates underscore the urgent need for technological advancements in postharvest handling. Such improvements are particularly crucial for avocados, as they would not only facilitate distribution but also improve storability, extend shelf life and increase usages after harvest. Additionally, avocados are climacteric fruits, characterized by their continued ripening after harvest because of high respiration rate and significant production of ethylene (6).

The pulp content in various avocado varieties ranges from 52.9 % to 81.3 % of the total fruit mass (7). Avocado pulp is notable for its high lipid and low carbohydrate levels after dehydration, resulting in high dry content. This makes avocado one of the few cultivated fruits where lipids are the primary component, comprising up to 25 % of the fruit's mass. The composition of avocado pulp typically includes 67-78 % moisture, 13.5-24 % lipids, 0.8-4.8 % carbohydrates, 1.0-3.0 % protein, 0.8-1.5 % ash, 1.4-3.0 % fiber and an energy density between 140 and 228 kcal (8).

Avocados have four times more nutritional value than most other fruits, except for bananas and they contain 1-3 % protein and significant amounts of fat-soluble vitamins (9). They are also rich in folic acid, calcium, potassium, magnesium, sodium, phosphorus, sulfur, silicon and vitamins E, B1, B2 and D (10). Avocados are particularly high in potassium, with 339 mg per 100 g, which helps regulate muscle activity and protect against cardiovascular diseases (11). Additionally, avocados are a good source of glutathione, a potent antioxidant that combats potentially carcinogenic compounds (12).

In addition, avocados rich in bioactive compounds, like phytosterols, are particularly abundant in the lipid fraction. The primary phytosterol in avocados is β-sitosterol (13). Diets rich in phytosterols can help reduce total cholesterol and LDL cholesterol levels. Furthermore, βsitosterol has special effects on immunity and has been shown to control cancer, HIV and infectious diseases. In cancer, it helps by suppressing carcinogenesis and it strengthens the immune system in HIV (14). β-sitosterol enhances lymphocyte proliferation and natural killer cell activity, which are important for inactivating invading microorganisms (15). Studies have also indicated that β-sitosterol can aid in weight loss by reducing compulsive eating and fat accumulation, especially in the abdominal area. In addition, avocados contain lutein, a carotenoid that provides protection against prostate cancer and eye diseases such as cataracts and macular degeneration (16).

The fully matured avocado fruits are consumed as such as a fresh cut fruit and smoothies. It is also processed

into various products, including frozen slices, packaged guacamole and avocado oil. As a climacteric fruit, avocados are optimally ripened post-harvest to maximize shelf life. In commercial settings, avocados are subjected to ethylene gas treatment to accelerate ripening prior to retail distribution, while consumers often expedite ripening by enveloping the fruit in newspaper or cover with brown paper bag and monitoring it daily. Once ripened, avocado pulp undergoes rapid deterioration after cutting, with accelerated browning due to oxidation catalysed by Polyphenol Oxidase (PPO) presenting a significant challenge in processing. To maintain quality, good avocado pulp should contain 98 % to 99 % avocado and only 1 % to 2 % additives. This necessity drives the development of new food processing methods that prioritize physical preservation processes over chemical ones.

In the current global economics, it is interested in trading food products between countries that emphasizes natural production methods and minimizes the use of chemicals in production and preservation. Freeze-drying technology is one of the best dehydration techniques to increase the shelf-life and allowing avocado to maintain its sensorial and nutritional characteristics.

To address these challenges, this study is designed to investigate the suitable drying technology of avocado fruit and analyze the fatty acid content of freeze-dried avocado fruit powder. This approach aims to extend the shelf life of avocados, making them available year-round and to assess their suitability of novel food products.

Materials and methods

The avocado fruit was purchased from Gudalur, Coimbatore district and the study was conducted in Community Science College and Research Institute, Madurai for standardization and analytical work.

Preparation of avocado fruit for making powder in freeze drying

Unripe avocados fruits were collected, covered with newspaper and kept at room temperature for ripening. The fruit weight ranged from 150 g to 500 g, with lengths varying between 13 cm and 48 cm and diameters ranging from 33 cm to 60 cm. The pH and total soluble solids of pulp averaged 6 - 7 and 3° Brix, respectively. Twenty-five (25 nos.) fully ripe avocados were washed, peeled and cubed. For freeze drying, avocado fruit cubes were subjected to freezing at -18° C for 48 hr in a domestic freezer. Avocado pulp samples were subsequently dried for 72 hr in a laboratory-scale vacuum freeze drying unit (Benhay SB-4, UK) under vacuum conditions (13.3 Pa) at a temperature of -44° C and a heating temperature of 24° C.

Physical properties of freeze-dried avocado fruit powder

Sample weight (g) was recorded using an Explorer Pro Balance, Model EP2102C (Ohaus Corporation, NJ, USA). Moisture content of the fresh and dried samples was determined using a Halogen Moisture Analyzer HB43-S (Mettler Toledo-AG, Zurich, Switzerland) set at 105° C. Water activity (aw) was measured using an Aqua Lab CX-2 1021

water activity meter (Decagon Devices Inco., Pullman, WA, USA). Sample pH was measured using an OAKTON 150 pH meter (OAKTON Instruments, IL, USA) and Total Soluble Solids (TSS) content measured using a Reichert AR200 digital hand-held refractometer (Reichert, Inc. NY, USA). Bulk density (g mL⁻¹), water solubility (per cent) and water absorption (per cent) were also determined by AOAC method. The freeze-dried avocado powder was depicted in Fig. 1.

Colour attributes of freeze-dried avocado fruit powder

Colour value was assessed in CR-410 Chroma Meter (Konica Minolta Sensing Americas, Inc., NJ, USA) employing Hunter values (L*, a*, b*). Hue angle (°) was calculated as presented in Eq. 1, with a correction of +180° for negative a* values (17)

Hue $(\circ) = 1 / \tan (b/a)$.

Nutrient content of freeze-dried avocado fruit powder

Crude protein content (%) of the dried powder was determined using the (18) Crude fat (%) (19), total dietary fibre (%) (18) and ash content (18).

FAME analysis

The fatty acids profile was determined by gas chromatography-mass spectrometry (GC-MS), through gas chromatograph with FID detector (CML_FOOD_GCFID1) brand AGILENT. The chromatographic conditions were as follows: injector temperature 250°C, detector temperature 25°C, oven temperature 140-230°C, Nitrogen was the carrier gas. A standard FAME 37 (C4-C22) was used. The fatty acids were determined through the official method (18). All the determinations were performed in triplicate (Fig. 2).

Statistical analysis

All experiments were carried out in triplicate and data were presented as mean \pm standard deviation. ANOVA table was used to statistically analyse the data (Version 17.2 windows) to find significant differences (p<0.05) between samples.

Shelf-life analysis of freeze-dried avocado fruit powder

Freeze dried avocado fruit powder was packed in airtight glass containers and stored in a room temperature for three months, during this period, to check the quality of fruit powder the basic analysis like moisture, water activity and objective measurements were carried out and sensory



Fig. 1. Freeze dried avocado fruit and powder.

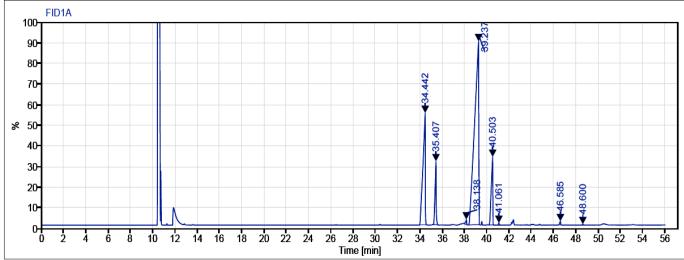


Fig. 2. Free fatty acid content of avocado fruit powder in FAME.

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attributes also recorded.

Results

Standardization of avocado fruit powder

Attempts have been made to develop avocado fruit powder for extending shelf life and make available throughout the year. Freeze-dried avocado cubes were in light yellowish green colour like fresh pulp. The freeze-dried cubes were light weight, spongy and puffed in appearance. The dried cubes maintained its original structure, shape and shrinkages were not noticed. The freeze-dried cubes were ground into a fine powder with good colour and texture. The yield 100 g of fresh avocado cubes were determined 70 % and peel weight was noticed 30 %. The yield of freeze-dried samples noted 17.5 %. Freeze drying technology creates a low temperature and water is removed by sublimation of ice under vacuum which is not only prevents enzymatic browning and produces a porous structure.

Physical properties of avocado fruit powder

Freeze dried powder was mixed with water at room temperature until a creamy thick consistency like that of the fresh avocado puree was achieved. Freeze dried powder rehydrated within 15 sec of mixing to produce a thick paste, with a puree-like consistency and vibrant green colour.

The water activity (a_w) of the fresh avocado pulp was found to be 0.9 and freeze-dried avocado sample 0.4. The bulk density value is important for characterizing, handling and processing of powders. The bulk density depends on both the density and the arrangement of the powder particles. As expected, freeze-dried powder had lower bulk density (0.2 g mL⁻¹). The fresh pulp was alkaline and drying resulted in a decline in pH and therefore an increase in acidity of the pulp. Soluble solids content of the dried samples was higher than the fresh puree due to the removal of water. When calculated on a dry matter basis the soluble solids content of freeze-dried powder was 2° brix. The values of physical parameters are presented in Table 1.

Colour value of avocado fruit powder

The colour attributes of fresh and dried avocado samples are given in Table 2. The Hue (°) values of the freeze-dried sample were like that of the fresh puree.

Table 1. Physical parameters of freeze-dried avocado fruit powder.

Physical properties	Fresh	Freeze dried avocado fruit powder
Water activity (a ^w)	0.9	0.4
Bulk density (g mL ⁻¹)	0	0.2
рН	7.6	6.7
TSS (°Brix)	1	2

Table 2. Colour attributes of fresh and dried avocado samples.

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Colour attribute	Fresh	Freeze-dried	
L*	62.3 ± 1.16b	82.96 ± 0.59a	
a*	-7.7 ± 0.21b	-14.32 ± 0.02a	
b*	$40.44 \pm 0.13b$	$60.38 \pm 0.29a$	
Hue (°)	$100.78 \pm 0.25a$	$103.34 \pm 0.07b$	

Proximate composition and nutrient content of freezedried avocado fruit powder

Based on the physical properties, freeze dried sample has been analysed for the nutrient content, free fatty acids and shelf-life studies. Compared with fresh and freeze-dried samples were significantly (p<0.05) higher in fat and protein total dietary fibre and ash content (Fig. 3).

Mineral content of avocado fresh and fruit powder sample

Mineral content of the freeze-dried avocado fruit powder was analysed and represented in Fig. 4. The results indicated significant levels of calcium (51.13 mg/100g), magnesium (87.21 mg/100g) and potassium (1198 mg/100g), iron (1.23 mg/100g) and sodium (5.1 mg/100g).

Fatty acid content of avocado fruit powder

Fatty Acid Methyl Ester (FAME) analysis revealed a total fat content of 44.98 %, with 31.05 % monounsaturated fatty acids (MUFA), primarily oleic acid (71 %), known for its cholesterol-lowering and heart health benefits. The powder also contained 98.70 % saturated fatty acids, 40.70 % polyunsaturated fatty acids (PUFA) (Table 3, Fig. 5).

Discussions

Freeze drying is a lyophilization process; it starts with frozen of material and subsequently dried in low temperature and low-pressure settings. In primary drying phase, water is

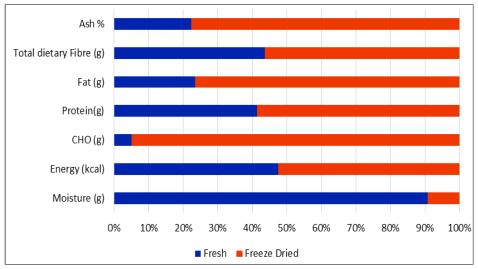


Fig. 3. Proximate composition and nutrient content of avocado fresh and dried samples.

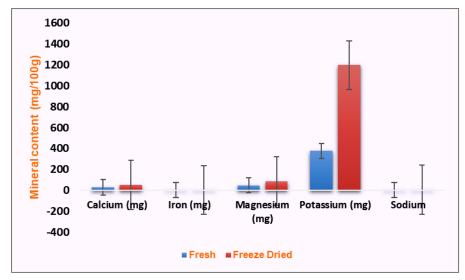


Fig. 4. Mineral content of avocado fresh and fruit powder sample.

Table 3. Free fatty acid content of avocado fruit powder.

RT [min)	Туре	Width [min]	Area	Height	Area percentage	Name
34.442	MM m	0.18	21503.39	1482.98	21.4175	C16:0 - palmitic acid
35.407	MM m	0.09	5960.29	841.27	5.9365	C16:1-palmitoleic acid
38.138	MM m	0.09	435.59	67.40	0.4339	C18:0 - stearic acid
39.237	MM m	0.31	63295.38	2428.16	63.0426	C18:1 cis - oleic acid
40.503	MM m	0.12	8700.95	902.07	8.6662	C18:2 cis - linoleic acid
41.061	MM m	0.06	119.52	29.28	0.1190	C20:0 - icosanoic acid or arachidic acid
46.585	MM m	0.08	333.12	51.75	0.3318	C20:3 cis - eicosatrienoic acid
48.600	MM m	0.06	52.67	12.00	0.0525	C22:2 -docosadienoic acid

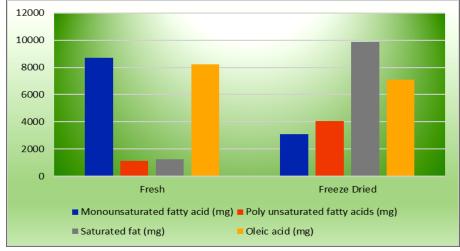


Fig. 5. Fatty acid content of avocado fruit powder.

removed through sublimation as the ice is converted to vapour, by the application of a minimal heat. The secondary drying phase involves the removal of unfrozen water molecules as the temperature is elevated. The impact of freeze drying on the chemical composition of avocado pulp was studied in previous experiments (20). They observed freeze drying resulted in minor alterations in pulp nutritional quality and concluded freeze drying was optimal for preserving shelf life and maintaining the sensorial and nutritional characteristics of the pulp. Freeze dried Hass avocado had a shelf life of up to 9 months with moisture content below 2.5 % in previous scientific experiments (21). The avocado powder obtained from grinding the puree has higher moisture content at saturation (2.59 g/g DM in 60 min) than other geometries of slices and cubes (22). As per FSSAI, a water activity less than 0.6 are required for fruits and vegetables powder products to maintain the quality.

The appreciable amount of calcium, iron, magnesium, potassium and sodium content has been found in freeze dried avocado fruit powder. The fruit stands out on potassium levels (339 mg $100~{\rm g}^{-1}$) when compared to other fruits, which regulates muscle activity and protects the body from cardiovascular diseases (11)

In comparison to other fruits, avocado fruit and fruit and powder contains high levels of monounsaturated fatty acids (oleic and palmitoleic acids), low polyunsaturated fatty acids (linoleic acid) and high levels of saturated fatty acid (palmitic and stearic acids). This fatty acid composition is influenced by the cultivars, maturity stage, anatomical region of the fruit and geographic location for plant growth (7).

Avocado oil extracted from the varieties Wagner,

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Fortuna, Hass and Fuerte had higher levels of Monounsaturated Fatty Acid (MFA) ranging from 59 to 72 % of total fatty acids, followed by saturated fatty acids (SFA), from 17 to 23 % and polyunsaturated fatty acids (PUFA) to a lesser extent with levels ranging between 10 and 14 % in earlier experiments (23). The influence of different pulp drying procedures on the fatty acid profile of Fortune variety of avocado was evaluated in earlier experiments (24). The authors reported, oleic fatty acid represented more than half of the total fatty acids of raw material verified that the dehydration of the pulp can affect the fatty acid profile.

Palmitic acid exerts multiple fundamental biological functions at cellular and tissue levels and its steady concentration is maintained by its endogenous biosynthesis through DNL, a metabolic pathway presents from the lower steps of the evolutionary scale. C18:0, also known as stearic acid, is a saturated fatty acid found in meat, dairy and some seeds.

It has several nutritional and other applications, including it may decrease the risk of cardiovascular and cancer disease. Stearic acid can promote glutamate uptake dose-dependently in cerebral neurons (26). C18:0 does not elevate serum LDL cholesterol concentrations, unlike other saturated fatty acids. This may be attributed to the body's rapid conversion of C18:0 into oleic acid. C18:1 cis fatty acid, also known as oleic acid, is the largest monounsaturated fat in the human diet. Monounsaturated fat consumption is associated with decreased low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein (HDL) cholesterol. In digestibility process, the amount of cis-9 C18:1 in a diet can increase the digestibility of total, 16-carbon and 18-carbon fatty acids.

Oleic acid consumption may help prevent rheumatoid arthritis by increasing leukotriene A3 levels, which inhibit pro-inflammatory LTB4. Oleic acid has decreased the total plasma cholesterol level; however, it has not changed plasma triglycerides and non-esterified fatty acids (27). Oleic acid may also help to control coronary heart disease and cancer.

C18:2 cis, knowing linoleic acid (LNA), is a polyunsaturated fatty acid (PUFA) that is an essential fatty acid in human nutrition due to its inability to be synthesized by humans. Linoleic acid is used for growth and development in skin cell membranes. Consumption of LNA may reduce the risk of cardiovascular disease and diabetes. Furthermore, LNA intake may decrease total blood cholesterol and low-density lipoprotein levels. Foods with very low-fat content (vegetables, fruits and grains) are predominantly rich in linoleic acid as the major PUFA (28). C20:3n-3, known as eicosatrienoic acid, is a polyunsaturated omega-3 fatty acid that exhibits numerous nutritional benefits.

Eicosatrienoic acid may contribute to cardiovascular protection through vasodilation, blood pressure reduction and atherosclerosis prevention. While eicosatrienoic acid is an essential component of human health, the body lacks the ability to synthesize it, necessitating dietary intake. Eicosatrienoic acid can be synthesized from ingested α -

linolenic acid or obtained through the consumption of fish or fish oil (29).

Removal of moisture through lyophilisation technique improve the maximum retention of nutrient content and free fatty acid content when it compared with fresh avocado fruit.

Conclusion

Avocado presents a viable alternative for the food industry, particularly in powder and oil processing. This study demonstrated the suitable drying methodology of avocado fruit without any changes as compared with fresh fruit. Physical and nutrient content of freeze-dried avocado fruit powder indicated the maximum retention of nutrients after drying of avocado fruit. FAME analysis of fatty acid profiling proved the maximum retaining of fatty acid content in freeze dried avocado fruit powder, hence the consumption of freeze -dried avocado fruit powder reducing cholesterol levels and mitigating the risk of cardiovascular diseases. Moreover, this fruit powder is used to develop nutraceutical value added foods and incorporation in pharmaceutical and cosmetic industries. Hence, this is the apt situation to consider diversity of plant species and it facilitates widespread cultivation and ensures consistent fruit availability throughout the year. With the increasing interest in research of avocado, enhance the production and utilization of this avocado fruit in India.

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Authors' contributions

JK Formulated research proposal, conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, original draft, review and editing. GG performed the roles of formal analysis, methodology part, resources, supervision, validation and visualization. SE conducted data curation and supervision. NL conducted the review and editing. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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