Comparative Study of Fatty Acids Profiles in *Mangifera indica* L. Seed Butter Extracts from Different Regions in Nepal Using GC-MS

Indu Bhatta, Prabina Shrestha, Prajwal Acharya, Sabina Pantha, Rojina Devkota & Achyut Adhikari*

Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal *Email: achyutraj05@gmail.com

Abstract

Mango is widely consumed for its pulp; however, the kernels are often discarded as a waste product despite their potential application. This research aims to study the potential application of the kernel in the production of butter. The study involves chemical profiling of butter extracted from kernels of mango collected from various regions of Nepal for their possible application in the confectionery industry as a cocoa butter replacement. Samples were collected from different districts, including Bardiya, Doti, Kathmandu, Tanahun, and Dhangadhi. The collected samples were cleaned, dried, and ground into powder. Butter was extracted employing the Soxhlet extraction technique using n-hexane as a solvent. The extracted mango seed butter was then analyzed using Gas Chromatography- Mass Spectrometry (GC-MS) to determine the fatty acid composition. Despite species-specific variations in concentration, octadecanoic acid (stearic acid), a crucial component of mango seed butter, was the most consistently found chemical in the samples studied. Some other important fatty acids such as oleic acid and palmitic acid were also present in the kernel of mango.

Keywords: Cocoa butter, Fatty acids, Mango kernel, Octadecanoic acid

Introduction

Mango (Mangifera indica L.) has been commercialized globally in more than 90 nations. It is commonly grown in tropical and subtropical regions of the world (Akhter et al., 2016). The growing global demand for mangoes and their processed products focuses on pulp for making juices, jams, and other items (Tharanathan et al., 2006). Consequently, mango seeds, a major byproduct along with peels, account for 60% of the fruit and are discarded as waste. Despite this, mango seeds, containing 7.3-15% fat, can produce over 123,000 metric tons of oil (Abdalla et al., 2007). Mango seed contains a type of fat called mango kernel fat (MKF) (Solís-Fuentes & Durán-de-Bazúa, 2020). Mango kernel fat is a rich source of stearic and oleic acid (Wu et al., 2015). These fats are notable for their distinct physical and chemical properties, which make them highly suitable for manufacturing confectionery products (Naeem et al., 2019). Consequently, mango seed lipids have gained attention for their potential as a cocoa butter

replacement. Where, four major fatty acids that include palmitic acid (24.4 %), stearic acid (33.6 %), oleic acid (37.0 %), and linoleic acid (C18:2, 3.4 %) account for more than 98 % of the total fatty acids in commercial cocoa butter (Lipp et al., 2001). Recently, studies have displayed the potential of mango as an alternative to cocoa butter with wide applications like pharmaceuticals, cosmetics, etc. due to its healthy fatty acids, antioxidants, and antimicrobial activity. Studies have shown that the main fatty acids found in mango kernel oil are about 45 % oleic acid and 38 % stearic acid (Wu et al., 2015). Oleic acid is an 18-carbon monounsaturated fatty acid, essential in human nutrition, and helps reduce triglycerides, LDL-cholesterol, total cholesterol, and glycemic index (Kittiphoom & Sutasinee, 2013). Also, the increase in stability over the oxidation of vegetable oil is attributed to oleic acid (Anwar et al., 2007). In addition to that, mango kernel seed extract also enhanced the oxidative stability of fresh-type cheese and ghee and extended their shelf life (Melo et al., 2019).

Cocoa butter is a light yellow fat that is obtained from the cocoa bean of the cocoa plant (*Theobroma cacao* L.). It is highly demanded by the food, pharmaceutical, and cosmetic industries. Moreover, it is unique among vegetable fats due to its composition and crystallization behavior. Cocoa butter contains 33.5% oleic acid, 25% palmitic acid, and 33% stearic acid, which accounts for above 80% of the total fatty acids in commercial cocoa butter (Chen et al., 1989; Shekarchizadeh et al., 2009). However, the challenges in cultivation, limited availability, and high demand contributed to the expensive nature of cocoa butter (Darmawan & Mutalib, 2024).

While the chemical composition of mango seed butter has been explored to some extent, there is a noticeable gap how this composition varies across different locations. The primary objective of this research is the extraction of the butter from the mango kernel sourced from various geographical regions and its analysis for fatty acid composition, to understand how the plant origin affects the composition of kernel fat. The study also aims to compare the composition of cocoa butter with that of mango kernel butter to explore its potential as a viable alternative. The findings indicate significant properties that highlight its suitability as an alternative to cocoa butter and promising application in the confectionery industry. Ultimately this research aims to bridge the gap by conducting a comparative study of kernel fat and the impact of geographical diversity on its chemical composition.

Materials and Methods

Sample collection

Mature fruits of sample *M. indica* were collected from different parts of Nepal (Bardiya, Doti, Tanahun, Kathmandu and Dhangadhi) to encapsulate a diverse range of Nepal's ecological zones. These locations represent different altitudes and climatic conditions from Tarai plain to the mid hills. Collected samples were identified by the botanist at the Central Department of Botany, Kirtipur, Kathmandu, Nepal. The collected samples were first washed, chopped, de-pulped, and again the kernel was chopped into

fine pieces, sun-dried, and crushed into fine powder. The obtained kernel powder was kept in air-tight container for further use.

Extraction of oil from kernel powder

In the thimble of the Soxhlet apparatus, 60 gm of coarse powder was suspended, and about 300 mL of n-hexane was poured into the round-bottom flask. The Soxhlet apparatus was set up, heated at 70°C, and allowed to stay for 8 hours under continuous extraction. At the end of the extraction, the resulting mixture containing the oil was distilled off to recover the solvent from the oil. The solvent was evaporated using a rotary vacuum evaporator and then dried in an oven at 45°C for 2 hours. The extracted fat was weighed and stored at 20°C until further analysis. The total yield obtained is expressed in percentage by using the following equation to determine the total fat yield of 100 gm of kernel powder on a dry weight basis and was expressed as a percentage:

$$Total\ fat\ yield = \frac{mass\ of\ extracted\ kernel\ fat}{Mass\ of\ mango\ kernel\ powder} \times 100$$

GC-MS analysis of mango kernel butter

The Gas Chromatography-Mass Spectrometer (GC-MS) analysis of mango kernel butter was performed at the Department of Food Technology and Quality Control, Nepal through GC-MS-QP 2010. The column used was RTX5MS and Helium as the carrier gas. The injection temperature was 220°C. The column oven temperature was maintained as follows: hold 80°C for 2 min, followed by an increase to 200°C for 3 min, and then up to 280°C for 4 min and the column head pressure was 67.7 kPa. The sample diluted with spectroscopic grade DCM (Dichloromethane) in a ratio of 1:10 was injected into the GC inlet maintaining a constant flow rate of 1.03 mL min⁻¹ and purge flow of 2 mL min⁻¹ in split mode using the split ratio of 15.0. The total flow was 18.5 mL min⁻¹. The Mass Spectrometry was performed at the scan speed of 1000 from 3 min up to 30.95 min. Identification of compounds was based on the retention indices determined by comparison of the mass spectral fragmentation patterns with those reported in the NIST 05 library.

Results and Discussion

Properties and percentage yield of sample

Butter was extracted from mango kernel powder collected from five different regions of Nepal using Soxhlet extraction. Among five different sample highest yield was obtained from IM2 (Doti) and lowest yield was obtained from IM4 (Tanahau). This may be due to the difference in moisture content and seed maturity among collected sample. The average percentage yield of the butter from mango kernel sample was calculated to be 7.022% (Table

1). The extracted mango kernel butter, derived from all places, was found to be highly viscous, solid, and sticky with a yellowish-white color.

Fatty acid composition of mango seed butter from different geographical reasons

The GC-MS analysis of mango seed butter showed the presence of varieties of fatty acids (Figure 1). The butter samples extracted from mango seeds from various regions of Nepal showed distinct fatty acid compositions (Table 2). The sample IM1 butter extracted from Bardiya district showed five

Table 1: Percentage yield of mango kernel butter

S.N.	Sample	Places	Product	Appearance	Color	Yield (%)	Average Percentage Yield
1	IM1	Bardiya				6.95	
2	IM2	Doti				7.2	
3	IM3	Kathmandu	Butter	Highly Viscous Solid and Sticky	Yellowish white	7	7.02%
4	IM4	Tanahun				6.88	
5	IM5	Dhangadhi				6.98	

Table 2: Fatty acid profile in mango seed butter of different varieties

Compounds (%)	Sample IM1	Sample IM2	Sample IM3	Sample IM4	Sample IM5
Octadecanoic acid	21.97	25.70	12.35	7.76	21.49
l-(+)-Ascorbic acid-2,6- dihexadecanoate	15.60	8.23	-	12.39	8.56
cis-9-Hexadecenal	-	=	52.71	43.68	50.50
2-Isoamyl-6-methyl pyrazine	5.73	2.10	11.48	-	5.13
Oxalic acid, 2-methyl phenyl octadecyl ester	-	5.99	-	-	9.67
6-Octadecenoic acid, (Z)-	47.42	-	-	-	-
Z,Z-4,6-Nonadecadien-1-ol acetate	9.27	-	-	-	-
Z-9-Pentadecenol	-	57.98	-	-	-
n-Hexadecanoic acid	-	-	7.82	-	-
1-Cyclopenta-2,4- dienylundec-10-en-1-one	-	-	15.64	-	-
Octadecanoic acid-2,3- dihydroxypropyl ester	-	-	-	14.91	-
2-Methyl-Z,Z-3,13- octadecadienol	-	-	-	6.07	-
9-Octadecenoic acid (Z)- methyl ester	-	-	-	-	4.65

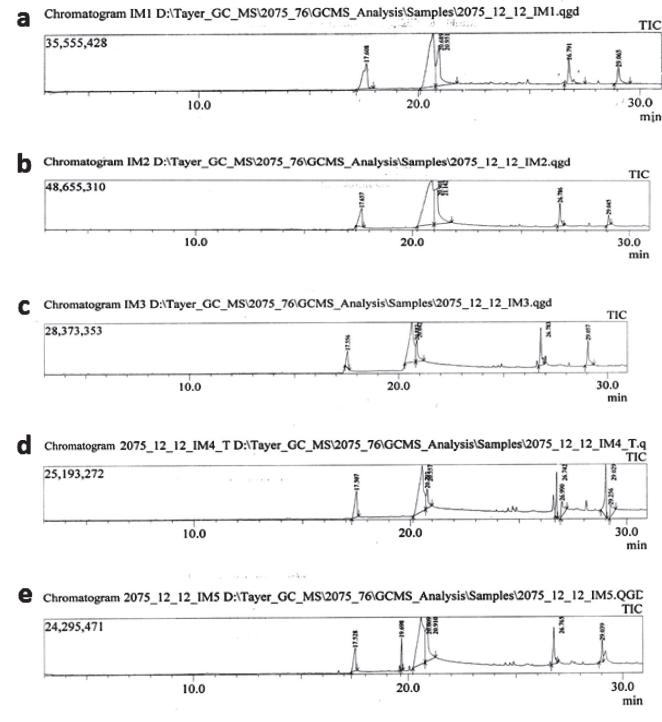


Figure 1: GC-MS chromatogram spectra of mango seed butter samples (a) IM1, (b) IM2, (c) IM3, (d) IM4, (e) IM5

different compounds with two major fatty acids, 6-octadecenoic acid (47.42%) and octadecanoic acid (21.97%), also known as stearic acid. The sample IM2 butter extracted from Doti showed 5 different compounds with octadecanoic acid i.e. stearic acid (25.70%) as predominant fatty acids. The sample IM3 from Kathmandu showed five different

compounds with major fatty acids: n-hexadecanoic acid i.e. palmitic acid (7.82%) and octadecanoic acid i.e. stearic acid (12.35%).

Five different compounds with major fatty acids were octadecanoic acid i.e. stearic acid (25.70%). The sample IM4 from the upper region of Tanahun

district in Nepal showed six different compounds with octadecanoic acid (7.76%) as a major fatty acid. The sample IM5 extracted from Dhangadi of Kailali district showed six different compounds with major fatty acids octadecanoic acid i.e. stearic acid (21.49 %) and oleic acid methyl ester i.e. 9- octadecenoic acid methyl ester (4.65%). All the varieties of the mango seed obtained from the different parts of Nepal showed octadecenoic acid but in different ratios. The constituents obtained were similar to those of the other research paper (Muchiri et al., 2012). The variation in the constituents is due to the cultivar, extraction technique, planting area, geographical, altitude, and the many different factors that elevated the constituents in the mango seed (Maldonado-Celis et al., 2019). The primary fatty acids found in mango kernel butter include stearic acid, palmitic acid, and 6-octadecanoic acid, while cocoa butter primarily contains stearic acid, oleic acid, palmitic acid, and linoleic acid (Apgar et al., 1987; Naik & Kumar, 2014). The fatty acid profile of mango kernel butter closely resembles that of commercial cocoa butter. Given the high demand and limited availability of cocoa butter, which contributes to its elevated price, mango kernel butter presents a promising, cost-effective alternative. It offers a high-quality option for replacing cocoa butter in various applications.

Conclusion

Mango (*Mangifera indica* L.) kernels sourced from various districts of Nepal was assessed for their fatty acid composition through GC-MS analysis. The results identified stearic acid, palmitic acid, and octadecanoic acid as the major fatty acid compounds in almost all sample. The difference in the percentage composition may be due to geographical variation and other factors like light, temperature, altitude, tree age, and other factors. Since cocoa butter and mango kernels have similar chemical constituent. mango kernel butter can be a suitable substitute in various application without compromising the desired characteristics of the end product. These findings suggest that mango kernel butter could be a viable and cost-effective alternative to the highdemand and expensive cocoa butter. However, further study must be conducted to know which variety of mango can be best alternative in terms of cocoa butter production, and how geographical condition affects the composition of the butter.

Author Contributions

I Bhatta conceptualized investigations, methodology, and data analysis and prepared the original draft. P Shrestha, P Acharya, S Pantha & R Devkota were involved in preparing the manuscript. A Adhikari helped in language editing.

Acknowledgments

We are thankful to the Department of Food Technology and Quality Control, Nepal for the GC-MS analysis.

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