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Comparative elucidation of functional metabolites in fermentation byproduct of *Nata de Saccharum* derived from sugarcane juice (*Saccharum officinarum* L.)

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Abstract

The present study offers a comparative elucidation of functional metabolites in fermentation byproduct of *Nata de Saccharum*, a biopolymer synthesized from sugarcane juice, using gas chromatography-mass spectrometry (GC-MS) analysis. The volatile compound profiles of both raw sugarcane (*Saccharum officinarum* L.) juice and its fermented counterpart were systematically analysed to explore the biochemical changes induced by microbial fermentation. The GC-MS chromatograms revealed significant qualitative and quantitative differences in metabolite composition between the two samples. In the hexane extract of sugarcane juice, predominant compounds included 2,4-di-tert-butylphenol, bis (2-ethylhexyl) phthalate, hexadecenoic acid methyl ester, pentafluoro propionic acid heptadecyl ester, and 6-octadecenoic acid methyl ester, among others. Hydrocarbons such as eicosane and heneicosane were detected in multiple retention times, suggesting natural variability in the volatile profile. In contrast, the fermented *Nata de Saccharum* extract exhibited a broader and more complex array of about 25 distinct compounds, such as dibutyl phthalate, linoleic acid ethyl ester, ethyl oleate, stigmasterol, and 13-hexyloxacyclotridec-10-en-2-one, a compound known for its antimicrobial, cytotoxic, and mosquito-repellent activities. Several new metabolites like dipalmitin, glycidyl palmitate, and high-molecular-weight fatty acid esters were detected only in the fermented sample, indicating microbial biotransformation during the fermentation process. These transformations suggest significant microbial enzymatic activity, resulting in lipid metabolism, esterification, and structural alterations. The emergence of ethyl esters and complex lipids contributes to the enhanced aroma, stability, and potential bioactivity of the fermented product. Moreover, comparative analysis shows variation in the abundance of common metabolites, which further underscores the dynamic biochemical shifts post-fermentation. The presence of C16 and C18 fatty acids, sterols, phthalates, and bioactive esters in *Nata de Saccharum* highlights the functional enhancement of sugarcane juice through fermentation. These alterations suggest potential nutraceutical and pharmaceutical applications, particularly due to the presence of compounds with anti-inflammatory, antioxidant, antimicrobial, and hypocholesterolemic properties, as supported by previous studies on fermented sugarcane derivatives and related functional foods. This study affirms that fermentation not only modifies the volatile profile of sugarcane juice but also enhances its functional metabolite content, thus establishing Nata de Saccharum as a promising fermented product with significant bioactive potential.

1. Introduction

Sugarcane (*Saccharum officinarum* L.) grows copiously in tropical regions of India and subtropics are also suitable for its cultivation. Sugarcane serves as primary raw material in sugar industry mainly. Raw material for refined sugar production is well known and cane wax has various pharmacological and cosmetic applications. The other important goods in unprocessed form of sugarcane includes, brown sugar, candy, syrup, toffee, gur, khandasari, molasses, and jaggery, sugarcane bagasse into xylitol (Felipe *et al*., 1997; Shaji *et al*., 2022), sugarcane scum as bioactive metabolites (Molina-Cortes *et al*., 2023). Yusof *et al*. (2000) reportedly made some relevant observations freshly made sugarcane juice, referred to as *Caldo de Cana* or *Garapa* in Brazil, is a well-liked non-alcoholic beverage that keeps for four days at 5°C. *Nuoc mia* or *mia da* known as sugarcane juice is quite common as a drink in Vietnam nowadays everywhere. Sugarcane juice mixed with lemon ferments rather quickly in Minya Egypt and is sold in juice shops nationwide in bottles (Kraig and Sen, 2013).

Sugarcane juice treats jaundice and various urinary ailments like dysuria or anuria quite commonly in rural India surprisingly enough. Sugarcane products from leaves juice and its byproducts contain fatty acids alcohol phytosterols terpenoids flavonoids glycosides and some phenolic acids obviously (Singh *et al*., 2015). Ornamental

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sugarcane with vivid red fleshed canes possessing antioxidant properties serves as fodder and vegetable quite effectively nowadays (Govindakurup, and Mohanraj, 2024). Sugarcane juice provides vital vitamins and minerals effectively for sustaining good health with its extremely nourishing plant-based properties. Implementing cost-effective tech essential for preserving freshness amidst fast deterioration somehow happens very suddenly these days. Sugarcane juice offers a natural energy boost quickly due to high sugar content especially for active people needing energy. Sugarcane yields numerous positive health impacts and its byproducts like bagasse can be converted into biofuel furthering sustainability quite remarkably (Zainuddin *et al*., 2025).

Silva *et al.* (2016) notably present their findings subsequently. Sugarcane juice is a low acidity drink with pH range of 4.5 to 5.5 with a highwater activity (Aw) of 0.99 and composed of approximately 80% water and 20% total dissolved solids. Sugarcane juice has a relatively low acidity with pH ranging roughly between 4.5 and 5.5 alongside highwater activity of 0.99. Sugarcane juice provides immediate energy quite rapidly in hot months across India and tastes amazing. This juice rehydrates bodily tissues quickly mostly because it consists largely of water and some sugar (Ogando *et al*., 2019).

Although sugarcane juice is a nutrient-dense product with numerous therapeutic benefits, its quick deterioration limits its processing and marketing. Properties of sugarcane juice get influenced by various factors like heat, enzymes oxygen and microorganisms quite significantly under certain conditions. Because simple sugars are present, it spoils soon after extraction. Shorter harvesting time to crushing, decontamination, and juice extraction processing periods thereby reduce the impact of microbial activity in the cane stalks and produce higher-quality juices. Juice color can also be impacted by the proliferation of microorganisms in addition to the previously established chemical and metabolic browning mechanisms (Oliveira *et al*., 2007). Chemical and enzymatic inversion also affects sugarcane juice quality; the main enzymes responsible for the juice's discoloration are polyphenol oxidase and peroxidase. Additionally, within a few hours following extraction, the juice becomes sour due to microbial fermentation, making it unsafe to drink. Heating can suppress the polyphenol oxidase enzyme. Ginger and lime are traditionally used to temporarily suppress enzymatic activity. Furthermore, to maintain the juice's original flavor during short-term preservation, anti-browning agents such citric acid and ascorbic acid are frequently used (Priyanka Chauhan *et al*., 2024). Hence, preservation by fermentation is an alternative technology to preserve the juice and to enhance the nutrient content with novel medicinal properties.

Nata is a fermented edible jelly like food derived from fermentation of sugars derived from fruits, juices or any sugar containing substrates. *Nata de coco* or bacterial cellulose or hydrogel-like polysaccharide produced from *Acetobacter xylinum* grown in coconut water (Phan *et al.,* 2023; Fei *et al.,* 2024). Cakar *et al*. (2014) highlighted that the production of *nata* or bacterial cellulose is a costlier venture to use the readily available fruit juices or sugar medium containing glucose or sucrose as carbon source. An alternate cost-effective medium and high *nata* yielding potential have been developed by Tyagi and Suresh (2016); Kamal *et al*. (2022); using the sugar industry waste namely molasses as the cheap sugar source (Devanthi *et al*., 2021). Fermented *Nata de Saccharum* from sugarcane juice is developed using static fermentation technology and comparatively evaluated the bioactive metabolites with GC-MS.

2. Materials and Methods

2.1 Selection of materials

For this study, the juice was extracted from Sugarcane variety CoC 25 (parentage Co 85002 and HR 83-144) released in the year 2017 by Sugarcane Research Station, Cuddalore, Tamil Nadu Agricultural University, Tamil Nadu, India (https://tnau.ac.in/site/research/varieties-released/).

2.2 Sugarcane juice extraction

Sugarcane was harvested quickly from fields and cleaned thoroughly free of dirt and debris with considerable effort afterward. They were subsequently pulverized quite thoroughly inside an unusually large double mill cane crusher powered by a rather hefty 10 HP motor. The juice obtained was allowed to settle for 15 min and pre-filtered using a double layered muslin cloth and pasteurized at 70°C for 1 min to kill the pathogenic microorganisms in juice and used for the preparation of fermentation media.

2.3 Microorganism and growth conditions

The *Komagataeibacter rhaeticus* B1 strain was isolated from sugarcane juice, sequenced using16S RNA approach and deposited as NCBI No: OQ581474. The culture was further identified and confirmed by ICAR-NBAIM, Kushmaur, Mau, Uttar Pradesh, India and deposited with strain designated as *K. rhaeticus* (NAIMCC TB 3976). This strain was used as mother culture for the preparation of sugarcane juice based *Nata de Saccharum*. Hestrin and Schramm medium containing 20 g/l glucose, 5 g/l peptone and yeast extract was used mostly (20 gl^{-1} glucose, 5 gl^{-1} peptone, 5 gl^{-1} yeast extract, 2.7 gl^{-1} Na_2HPO_4, 1.15 gl^{-1} citric acid. H_2O) (Hestrin and Schramm, 1954; Gayathry and Gopalaswamy, 2014).

2.4 GC-MS analysis of sugarcane juice and fermented *Nata de Saccarum*

A known quantity of sugarcane juice was extracted vigorously using hexane for separation of various compounds *via* liquid solvent extraction and left overnight to evaporate slowly. Bioactive metabolites from hexane solvent extract of sugarcane juice and *Nata de Saccharum* were elucidated using Perkin Elmer Clarus SQ8C GC-MS model equipped with non-polar DB5 MS capillary column at Tamil Nadu Agricultural University in Coimbatore, India (Sridharan *et al*., 2021). Injector port temperature was cranked up to 220°C and interface temperature got programmed as 250°C with source maintained rather steadily at 220°C. Oven temperature was programmed available at 75°C for 2 min then ramped quickly at 10°C min^{-1} upto 150°C and subsequently 250°C. Split ratio was configured at 1:12 and injector operated in splitless mode. An Agilent Co. USA purchased (DB-5 MS, capillary standard non-polar column) was employed for the elution with 0.25 mm OD and 0.25 µm ID with an approximate length of thirty meters. Carrier gas was helium at 1 ml min^{-1} very accurately. The MS scan range was set from 50 Da to 550 Da nominally. Source temperature remained relatively steady at 220°C under a motor vacuum pressure of roughly 4.5e-6 which was low. Ionization energy somehow stubbornly hovered around a rather unusually negative value of -70eV. Helium gas was utilized at 1 ml min^{-1} flow rate as carrier gas. MS had a pre-filter built in which significantly reduced neutral particles inside. Data system boasts inbuilt libraries that facilitate rather elaborate spectrum searching and matching with considerable precision obviously at great speed.

NIST MS Search 2.2v apparently contains over five lakh references. GC-MS mass spectrum was subsequently dissected thoroughly afterwards with great care and minute attention. Known components stored in inbuilt library were interpreted using vast database from National Institute Standard and Technology labelled NIST14 rather elaborately.

3. Results

3.1 GC-MS elucidation of sugarcane juice and fermented *Nata de Saccharum*

The details of molecules with their molecular weight and peak area % elucidated for both the Sugarcane juice extract and *Nata de Saccharum* extraction presented in Table 1 and 2. The graphical representation in Figures 1 and 2 depict volatile compounds present in sugarcane juice extract and *Nata de Saccharum* extract. Figure 3 illustrates the comparative depiction of GC-MS analysis identifying volatile compounds present in Sugarcane juice and *Nata de Saccharum*. The current investigation on GC-MS of sugarcane juice showed different metabolites in the hexane extract of sugarcane juice. The major peak area was occupied by 2,4-di-tert-butylphenol, bis(2-ethylhexyl) phthalate, hexadecanoic acid, methyl ester, pentafluoropropionic acid, heptadecyl ester, silane, trichloro-octadecyl, 6-octadecenoic acid, methyl ester. Eicosane and benzenepropanoic acid 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester and methyl 10-trans12-cis-octadecadienoate had area per cent around 3.0. Two compounds; namely, heneicosane and eicosane occurred multiple times at various minutes (Figure 3).

The y-axis lists the detected volatile compounds, while the x-axis visually compares their presence and relative abundance in both extracts. The color-coded horizontal bars correspond to the GC-MS peak area percentages, which reflect the proportion of each compound in the total volatile composition. This data provides a detailed chemical fingerprint of both extracts and highlights how the fermentation process alters the volatile profile of sugarcane juice, leading to the formation of *Nata de Saccharum*.

Table 1: Compounds elucidated for sugarcane juice using GC-MS

S.No.	Rt (min)	Area %	H%	A/H	Molecular weight and formula	Compounds elucidated
1	17.72	1.88	2.35	2.76	196, $C_{14}H_{28}$	1-Tetradecene
2	20.27	1.24	1.67	2.57	296, $C_{21}H_{44}$	Heneicosane
3	20.53	1.29	1.35	3.28	204, $C_{15}H_{24}$	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-
4	20.91	20.79	20.47	3.51	206, $C_{14}H_{22}O$	2,4-Di-tert-butylphenol
5	22.91	2.75	3.56	2.66	310, $C_{16}H_{29}F_3O_2$	Tetradecyl trifluoroacetate
6	23.09	2.15	2.79	2.66	352, $C_{16}H_{33}I$	Hexadecane
7	25.49	3.30	4.23	2.70	282, $C_{20}H_{42}$	Eicosane
8	27.61	2.54	3.01	2.91	266, $C_{19}H_{38}$	1-Nonadecene
9	29.10	1.79	1.68	3.68	278, $C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
10	30.16	5.34	5.31	3.47	386, $C_{18}H_{37}C_{13}$	Silane, trichlorooctadecyl-
11	30.44	8.88	9.50	3.23	270, $C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
12	30.60	3.28	3.08	3.68	292, $C_{18}H_{28}O_3$	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
13	31.88	5.97	3.60	5.72	402, $C_{20}H_{35}F_5O_2$	Pentafluoropropionic acid, heptadecyl ester
14	32.43	2.33	2.65	3.03	298, $C_{19}H_{38}O_2$	Isopropyl palmitate
15	34.02	3.36	2.89	4.01	294, $C_{19}H_{34}O_2$	Methyl 10-trans,12-cis-octadecadienoate
16	34.17	4.62	3.65	4.37	296, $C_{19}H_{36}O_2$	6-Octadecenoic acid, methyl ester, (Z)-
17	34.71	2.03	1.77	3.96	450, $C_{32}H_{66}$	Dotriacontane
18	34.81	2.34	1.98	4.08	298,$C_{19}H_{38}O_2$	Methyl stearate
19	36.30	2.43	2.42	3.48	312, $C_{20}H_{40}O_2$	Hexadecanoic acid, butyl ester
20	43.78	11.92	11.63	3.54	390, $C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate

Table 2: Compounds elucidated in *Nata de Saccharum* using GC-MS

S.No.	Rt (min)	Area %	H%	A/H	Molecular weight and formula	Compounds elucidated
1	22.89	1.36	2.07	2.78	238,$C_{17}H_{34}$	1-Heptadecene
2	30.40	4.71	3.15	6.32	230, $C_{12}H_{22}O_4$	Undecanedioic acid, monomethyl ester
3	31.10	2.48	2.72	3.85	278, $C_{16}H_{22}O_4$	Dibutyl phthalate
4	31.83	2.76	3.01	3.87	284, $C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
5	31.99	0.77	1.19	2.72	296, $C_{21}H_{44}$	Heneicosane
6	33.10	1.79	1.80	4.19	280, $C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one
7	33.44	33.49	31.06	4.56	280, $C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one
8	34.17	1.09	1.31	3.52	282, $C_{20}H_{42}$	Eicosane
9	35.65	1.18	1.44	3.44	308, $C_{20}H_{36}O_2$	Linoleic acid ethyl ester
10	35.81	1.42	1.40	4.30	310, $C_{20}H_{38}O_2$	Ethyl oleate
11	36.41	2.55	1.80	5.98	185, $C_{13}H_{15}N$	1-Cyano-4-cyclohexylbenzene
12	36.35	2.62	2.19	5.06	256, $C_{14}H_{28}O_2Si$	bis[(2Z)-Hex-2-en-1-yloxy](dimethyl)silane
13	36.67	1.11	1.33	3.54	282, $C_{20}H_{42}$	Eicosane
14	38.89	3.30	3.01	4.63	312, $C_{19}H_{36}O_3$	Glycidyl palmitate
15	42.17	5.49	5.88	3.95	332$C_{22}H_{20}OS$	2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide
16	42.54	1.80	1.92	3.97	234, $C_{17}H_{30}$	1,8,11-Heptadecatriene, (Z,Z)-
17	42.64	6.42	6.73	4.03	640, $C_{38}H_{76}O_5Si$	1,3-Dipalmitin, TMS derivative
18	43.15	1.53	1.87	3.46	380, $C_{27}H_{56}$	2-Methylhexacosane
19	43.44	4.20	3.41	5.21	330, $C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
20	43.76	4.36	5.47	3.37	390, $C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate
21	44.00	3.71	2.12	7.39	412, $C_{29}H_{48}O$	Stigmasterol
22	45.01	3.88	5.53	2.96	486, $C_{32}H_{54}O_3$	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,14
23	45.38	2.36	2.39	4.17	280, $C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one
24	45.81	1.74	2.32	3.17	284, $C_{19}H_{24}O_2$	2-Propenoic acid, 3-phenyl-, 1,7,7-trimethylbicyclo[2.2.1] hept-2-yl ester
25	45.89	3.88	4.87	3.37	274, $C_{14}H_{30}O_3Si$	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDMS

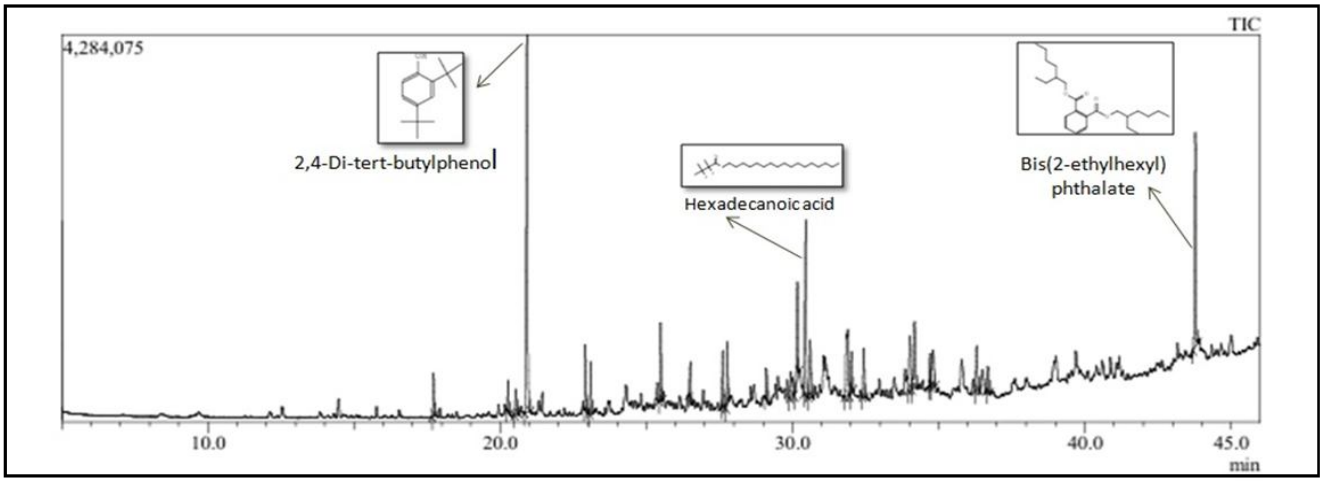

Figure 1: GC-MS elucidation of different phytoconstituents in sugarcane juice.

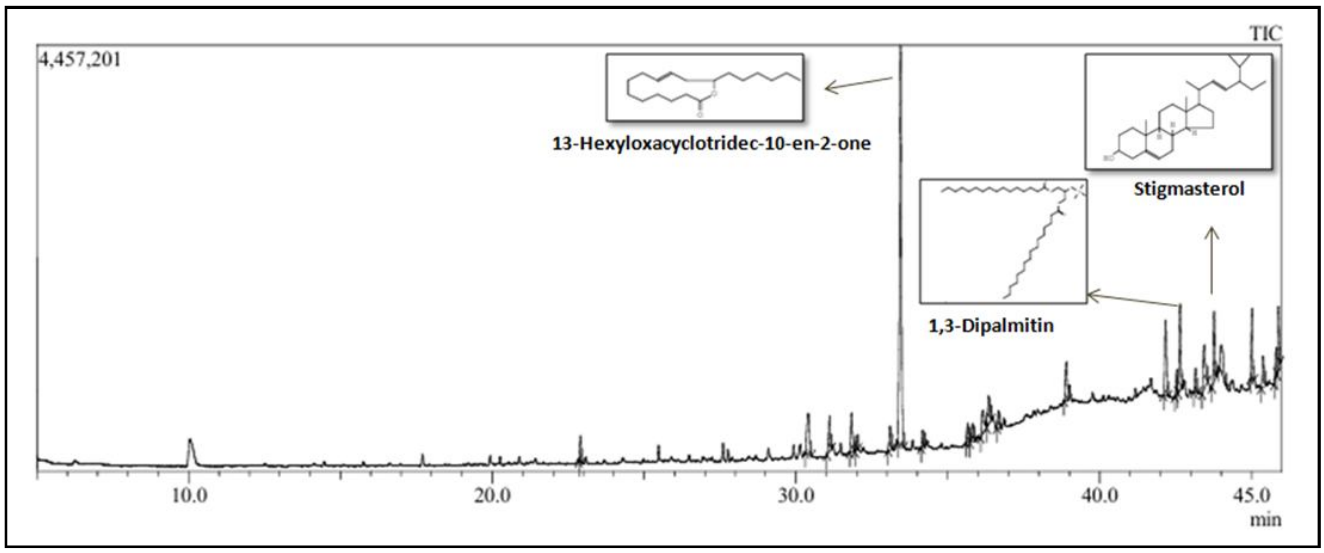

Figure 2: GC-MS elucidation of different phytoconstituents in *Nata de Saccharum*.

Table 3: Comparison of compounds in sugarcane juice and *Nata de Saccharum*

S.No.	Compounds in sugarcane juice	Compounds in *Nata de Saccharum*
1	1-Tetradecene	1-Heptadecene
2	Heneicosane	Undecanedioic acid, monomethyl ester
3	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-	Dibutyl phthalate
4	2,4-Di-tert-butylphenol	Hexadecanoic acid, ethyl ester
5	Tetradecyl trifluoroacetate	Heneicosane
6	Hexadecane	13-Hexyloxacyclotridec-10-en-2-one
7	Eicosane	Eicosane
8	1-Nonadecene	Linoleic acid ethyl ester
9	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Ethyl oleate
10	Silane, trichlorooctadecyl-	1-Cyano-4-cyclohexylbenzene
11	Hexadecanoic acid, methyl ester	bis[(2Z)-Hex-2-en-1-yloxy](dimethyl)silane
12	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4 -hydroxy-, methyl ester	Glycidyl palmitate
13	Pentafluoropropionic acid, heptadecyl ester	2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide
14	Isopropyl palmitate	1,8,11-Heptadecatriene, (Z,Z)-
15	Methyl 10-trans,12-cis-octadecadienoate	1,3-Dipalmitin, TMS derivative
16	6-Octadecenoic acid, methyl ester, (Z)-	2-Methylhexacosane
17	Dotriacontane	2-hydroxy-1-(hydroxymethyl) ethyl ester
18	Methyl stearate	Bis(2-ethylhexyl) phthalate
19	Hexadecanoic acid, butyl ester	Stigmasterol
20	Bis(2-ethylhexyl) phthalate	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,14
21	-	2-Propenoic acid, 3-phenyl-, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester
22	-	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDMS

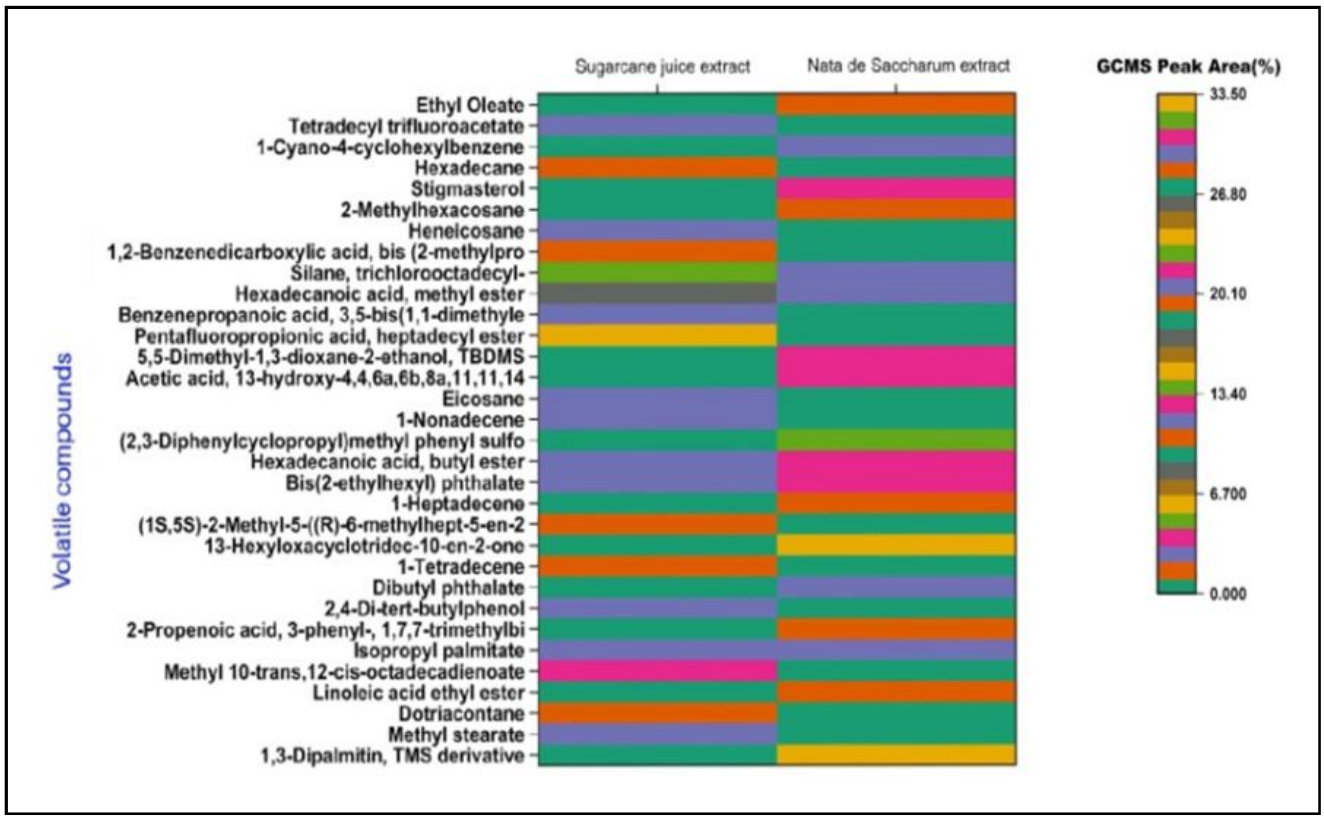

Figure 3: GC-MS Peak area % of different compounds elucidated in sugarcane juice and fermented *Nata de Saccharum*.

4. Discussion

The comparative elucidation of compounds in sugarcane juice and *Nata de Saccharum* using GC-MS is presented in Table. 3. In the present study, fermentation of sugarcane juice with *K. rhaeticus* resulted in the production of intracellular signaling molecule, namely; dipalmitin a diacylglycerol compound that contains saturated 16-carbon palmitic acid. Dipalmitin is reported as Palm oil fractionation that relies heavily on its peculiar templating effect for disarticulating fatty acid molecules within various food processing protocols (Da Silva *et al*., 2017). Similar type of compounds elucidated in nata de saccharum evidentially proves that fermentation of sugarcane juice has liberated anti-inflammatory molecule namely palmitic acid. In *Nata de Saccharum,* about 25 different compounds were elucidated and they were heptadecene, undecanedioic acid, monomethyl ester, dibutyl phthalate, hexadecanoic acid, ethyl ester heneicosane, 13-hexyloxacyclotridec-10-en-2-one, eicosane, linoleic acid ethyl ester, ethyl oleate, 1-cyano-4-cyclohexylbenzene, bis[(2Z)-Hex-2-en-1-yloxy] (dimethyl)silane eicosane, glycidyl palmitate, (2,3-diphenylcyclopropyl)methyl phenyl sulfoxide,1-heptadecatriene, (Z,Z)- 1,3-dipalmitin, TMS derivative, 2-methylhexacosane, hexadecanoic acid, 2-hydroxy-1--(hydroxymethyl) ethyl ester, bis(2-ethylhexyl) phthalate, stigmasterol, acetic acid, 13-hydroxy, propenoic acid, 3-phenyl-, 7-trimethylbicy, 5-dimethyl-1,3-dioxane-2-ethanol.13-hexyloxacyclotridec-10-en-2-one exhibits potent antimicrobial activity against various bacteria including *Salmonella* and *Staphylococcus* with numerous high receptor binding sites (Singh *et al*., 2023), mosquito repellent (Paulraj *et al*., 2021) and chemotherapeutic/ cytotoxic activity against the cancer cells (Dirar *et al*., 2014). The presence of this novel compound in *Nata de Saccharum* is triggered due to metabolic activity of the bacterial fermentation of sugarcane juice.

Sugarcane juice is more vulnerable to bacterial and yeast contamination soon after crushing due to which discolouration and unappealing odour develops in a short period of storage. Fermenting juices with bacteria is a novel nutritional strategy with possible health advantages (Saud *et al*., 2024). The bacteria used in the present study is reported to have probiotic properties which lead to biotransformation that alters the profile and type of bioactive molecules, while also improving organoleptic characteristics for long duration storage. Hence, it is justifiable that the present investigation has positively explored the method of preservation of sugarcane juice by fermentation into *Nata de Saccharum* and authenticates the comparative evaluation of both the products by means of metabolites elucidation using GC-MS.

The results show that while some compounds are present in both extracts, their relative concentrations differ, indicating significant biochemical transformations. The presence of fatty acids, esters, phthalates, hydrocarbons, and sterols in varying proportions suggests bacterial metabolism by enzymatic activity affecting the sugarcane juice during fermentation. For instance, the presence of ethyl oleate, hexadecane, and heptadecane contributes to the distinct aroma and physicochemical properties of the final product. Detection of higher molecular weight compounds like palmitates sterols and phthalates in *Nata de Saccharum* suggests formation of new structural components not prominent in sugarcane juice previously. Some compounds, such as pentafluoropropionic acid esters, benzene

derivatives, and phthalates, are often associated with fermentation byproducts or secondary metabolites produced by microbes during the transformation process. The presence of C16 and C18 fatty acids and their esters suggests lipid metabolism, which may play a role in the structural integrity of *Nata de Saccharum*. The significant chemical variations between the two extracts demonstrate that microbial fermentation of sugarcane juice has a profound impact on the volatile composition, potentially altering the flavor, aroma, and functional properties of the final product. Tiwari *et al*. (2023) have used hyperlipidimic rats to prove the pharmacological benefits in lowering the triglycerides and compounds using GC-MS in ethanolic and aqueous extracts of *Allium sativum*. Scum extracts possess cane wax, polyphenols, and flavonoids water-soluble vitamins and proteins with more antioxidant and radical scavenging activity (Lu *et al*., 2017). Cane juice often appears opaque and ranges in colour from brownish hues gradually deepening into dark emerald green shades. Colour impacts consumer approval due largely to presence of anthocyanins and other compounds formed from alkaline breakdown of fructose in sugarcane. Enzymatic browning by polyphenol oxidase and peroxidase and oxidation of chlorophyll and polyphenols often cause juice browning after extraction (Prati *et al*., 2005). Chauhan *et al*. (2002) demonstrated considerable prowess in juice production thereby showcasing their unusually high aptitude quite effectively investigated eight sugarcane cultivars cultivated in India. Samples were examined physicochemically and sensory, and juice yields were noted. In that investigation, the cane juice was pasteurized after additives were added.

According to the findings of Rajendran *et al*. (2017a, b) using FTIR spectroscopy and GC-MS analysis, sugarcane juice serves as a great beverage that may be utilized as a medicinal and functional food substitute for a number of illnesses. They identified a total of 14 compounds such as alkaloids, glycosides, phenols, saponins, tannins, and sugars through phytochemical examination. Cyclopropyl 4 methoxyphenyl ketone and 5-hydroxymethylfurfural were the two main chemicals found. Minor compounds including pentanal, 5-(methylene cyclopropyl) and isopropyl linoleate were also elucidated. Nutritional advantages abound in juice extracted from Co 86032 sugarcane variety dubbed wonder cane owing largely to presence of saponins and glycosides. Rohit *et al*., (2024) identified roughly 16 crucial elements alongside 25 distinct volatile compounds and numerous bioactive phytochemicals. While in the present findings, sugarcane juice shows the presence of alkanes, alkene, acids, esters, phthalates, and even inorganic silane molecules.

Rajendran *et al*. (2017b) investigated shelf life of sugarcane juice with natural preservatives like ginger and neem and conducted GC-MS analysis revealing 5-hydroxymethylfurfural as predominant compound in raw juice at 39.56% whereas treated juice contained compounds like 9 12 15-octadecatrienoic acid and 8H-pyrano [3 4 b] pyrimido [5 4-d] furane exhibiting antimicrobial antifungal anticancer antioxidant and antimutagenic properties effectively. While in the present study, elucidation of the sugarcane juice using GC-MS strongly exhibited higher peak area for 2,4-Di-tert-butylphenol which is also a phenolic compound possessing anti-inflammatory properties. But these phenols are highly unstable and deform during storage, hence fermentation into another form of product, namely *Nata de Saccharum* will make the value addition possibilities of sugarcane juice.

Aparna *et al*. (2012) reported various activities of cane juice including anti-inflammatory and hypocholesterolemic and cancer preventive properties quite effectively. Researchers such as Chen *et al*. (2020) conducted studies and apparently identified forty-five volatile organic compounds and various phenolic compounds from fermented sugarcane juice. Researchers apparently identified forty-five volatile organic compounds and sundry phenolic compounds from fermented sugarcane juice somehow. Furfural benzene acetaldehyde and 2,3-butanedione are among key aroma-active components in cane juice derived brown sugar significantly impacting its aroma (Serafim and Lanas, 2019; Liu *et al*., 2021). The findings of current research are in accordance with the above findings and substantially support the presence of similar type of volatile bioactive compounds in sugarcane juice.

Hexadecanal and eicosenoic acid along with octadecanoic acid were found in chewing canes from Sirukamboor's collection in Tamil Nadu India (Shanmuganathan *et al*., 2023). In the present study, the presence of fatty acids, esters, phthalates, hydrocarbons, and sterols in *Nata de Saccharum* suggests that microbial metabolism and enzymatic activity converted the sugarcane juice into a different form of edible food product that is made purely of cellulose, chewy and tender for its use in food adjuvants and fillings.

5. Conclusion

The present investigation underscores the significant transformation that occurs in sugarcane juice upon microbial fermentation, leading to the development of *Nata de Saccharum*, a novel fermented product with enhanced bioactive properties. Sugarcane juice, traditionally recognized for its refreshing taste, immediate energy boost, and therapeutic benefits, is highly perishable due to enzymatic browning and microbial spoilage. Fermentation using *K. rhaeticus* emerges as a promising biotechnological approach to overcome this limitation while simultaneously enriching the juice with functional metabolites. Through GC-MS analysis, this study demonstrated the marked biochemical shift between raw sugarcane juice and its fermented counterpart. The presence of compounds such as palmitates, phthalates, fatty acid esters, and bioactive sterols in *Nata de Saccharum* not only reflects microbial metabolic activity but also reveals the potential of fermentation to improve the therapeutic and nutritional value of sugarcane-derived products. Notably, compounds like 13-hexyloxacyclotridec-10-en-2-one and dipalmitin, which possess antimicrobial, anticancer, and anti-inflammatory properties, were elucidated in the fermented extract, suggesting added functional benefits. Furthermore, the transformation of volatile and non-volatile components during fermentation indicates potential flavor, aroma, and stability improvements in the final product. This conversion highlights a valuable avenue for utilizing sugarcane juice, especially in regions where it is abundantly available but poorly preserved. Overall, the study reinforces the role of fermentation as a sustainable and cost-effective strategy for enhancing the shelf life, nutritional profile, and medicinal properties of sugarcane juice.

Future work for research

The development of *Nata de Saccharum* not only diversifies sugarcane-based products but also adds value to the sugar industry by potentially utilizing its waste streams for high-value functional foods especially in preserving the highly perishable sugarcane juice

by fermentation. Future work may explore clinical applications and sensory profiling to better position fermented sugarcane products in the functional beverage market.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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