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Effect of Processing Level on Metabolite Profiles and Antioxidant Activity of Palm Sugar Using Untargeted LC–MS

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A B S T R A C T

Palm sugar derived from sugar palm sap (*Arenga pinnata* Merr.) is widely used as a natural sweetener and contains various minor compounds that may contribute to its functional properties. Differences in processing intensity during the production of liquid, moulded, and granulated palm sugar may induce chemical transformations that affect metabolite composition and antioxidant activity. However, comparative information linking processing level with these changes remains limited. This study evaluated the effects of different processing levels on the chemical characteristics and DPPH radical scavenging activity of palm sugar products. Antioxidant activity was determined using the DPPH method at a concentration of 1000 ppm, while metabolite profiling was conducted using untargeted LC–MS/MS Q-TOF in positive ion mode over an m/z range of 50–1200. Untargeted LC–MS/MS combined with principal component analysis (PCA) indicated differences in metabolite profiles among products, with liquid palm sugar showing the highest number of tentatively annotated features, followed by molded and granulated palm sugar. Increasing processing intensity was associated with shifts in metabolite composition, including reduced occurrence of oligosaccharide-related compounds and tentative detection of monosaccharide-related and heat-derived compounds such as maltol and phenolic-type derivatives. DPPH radical scavenging activity increased with processing level, reaching 39.23%, 55.93%, and 63.76% for liquid, molded, and granulated palm sugar, respectively. These findings suggest that thermal processing alters the chemical profile of palm sugar and may enhance its antioxidant properties under the conditions studied.

Contribution to Sustainable Development Goals (SDGs):

SDG 3: Good Health and Well-being

SDG 9: Industry, Innovation and Infrastructure

SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Palm sap (*Arenga pinnata* Merr.) is a natural liquid obtained from sugar palm trees. It has long been utilized in traditional food products, particularly alcoholic beverages and conventional palm sugar [1]. The sap is characterized by high water and sugar

content, predominantly sucrose, together with minor amounts of proteins, minerals, and other constituents [2]. Due to its natural origin and relatively low glycemic index, palm sap and its derivatives have attracted increasing interest as alternative sweeteners with potential nutritional benefits [3].

Palm sugar is one of the major derivative products of palm sap and is recognized for several advantages over refined cane sugar, including lower glycemic index and caloric content [4,5]. In addition to carbohydrates, palm sugar contains minor



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nutritional components, including organic acids, minerals, amino acids, and phenolic compounds, which contribute to its distinctive colour, flavour, and antioxidant potential [3,6,7]. These characteristics make palm sugar suitable for broader food applications compared with conventional sweeteners.

In practice, palm sap can be processed into several forms of palm sugar, including liquid, moulded, and granulated. These products differ mainly in heating intensity, final soluble solid concentration ($^{\circ}$ Brix), and mechanical treatment during cooling and crystallization [8]. Liquid palm sugar offers easy dissolution and flexible use, whereas molded and granulated forms provide better handling, packaging, and storage stability [9].

Thermal processing involved in palm sugar production not only concentrates sugars through water evaporation but also triggers chemical reactions such as sucrose degradation, caramelization, and the Maillard reaction [10,11]. These reactions may modify chemical composition and generate new compounds that influence color, flavor, and antioxidant properties [12]. However, comprehensive information regarding how different processing levels affect antioxidant activity and metabolite composition among various palm sugar products remains limited. Therefore, further investigation is required to understand processing-induced chemical transformations in palm sugar.

1.2. Literature Review

Previous studies have shown that palm sap contains various bioactive compounds whose composition may vary depending on geographical origin and agronomic factors. Relatively high antioxidant activity and phenolic content have been reported in palm sap from different production regions, indicating its potential as a functional food ingredient with added value [13].

Several studies have also highlighted the nutritional advantages of palm sugar compared with refined sugar, especially its lower glycemic index and the presence of beneficial minor compounds such as phenolics, amino acids, and minerals [3,4]. These compounds may contribute to antioxidant activity and improved nutritional quality.

Processing conditions are known to strongly influence the final quality of sugar products. Heating treatments can promote sucrose hydrolysis, caramelization, and Maillard reactions, leading to the formation of compounds that affect sensory and functional properties [10,11]. Despite these findings, most previous studies have focused on single palm sugar products or simple comparisons with other sweeteners.

Recent developments in untargeted liquid chromatography–mass spectrometry (LC–MS/MS) have enabled broad metabolite profiling without prior selection of target compounds, making this technique highly suitable for studying complex chemical changes during food processing [14]. Nevertheless, the application of untargeted LC–MS/MS to compare palm sugar products produced under different processing levels has rarely been reported.

1.3. Research Objective

This study aimed to evaluate the effects of different palm sap processing levels on the chemical and functional characteristics of liquid, molded, and granulated palm sugar. Specifically, antioxidant activity was determined using the DPPH radical scavenging assay, and changes in metabolite profiles were analysed using an untargeted LC–MS/MS approach. The findings of this study are expected to provide insights into processing-

induced chemical transformations in palm sugar and support the development of palm sugar products with improved functional quality.

2. MATERIALS AND METHODS

This study was conducted between October and December 2025 at Universitas Andalas (Padang, Indonesia) and the Chemistry and Bioprocess Laboratory, National Research and Innovation Institute (BRIN), Serpong, Indonesia

2.1. Materials and Instruments

Palm sap was obtained from sugar palm trees in Nagari Labuah Gunuang, Lima Puluh Kota Regency, West Sumatra, Indonesia. Analytical-grade methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), and formic acid were used for antioxidant and LC–MS analyses. Mobile phases for LC–MS consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Cooking oil and distilled water were used during palm sugar processing.

LC–MS analysis was performed using an Agilent Revident LC/Q-TOF system. Antioxidant activity was measured using a Shimadzu UV-1800 spectrophotometer. Supporting laboratory equipment included an ultrasonic bath, a vortex mixer, an analytical balance, a freezer, palm sugar moulds, and a 20-mesh sieve.

2.2. Research Procedures

2.2.1. Palm Sugar Preparation

Palm sap was obtained by direct tapping of sugar palm trees owned by local farmers in West Sumatra. Fresh sap was filtered to remove impurities and processed into palm sugar within ± 90 minutes after tapping.

To produce liquid sugar, palm sap was heated until water partially evaporated, causing foaming and a yellowish colour. Cooking oil was added in a small amount to limit foaming. Heating was continued with occasional stirring until the total soluble solids reached approximately 70 $^{\circ}$ Brix, after which heating was stopped, and the liquid palm sugar was cooled and packaged.

To produce moulded sugar, liquid palm sugar was further heated with continuous stirring until viscosity increased, yielding a brown concentrate with a total soluble solids content of approximately 90 $^{\circ}$ Brix. Foam and fine impurities were removed during heating. The viscous sugar was briefly cooled, poured into molds, air-dried for 1–2 h until hardened, and then packaged.

Granulated palm sugar was obtained by further heating the viscous sugar to approximately 96 $^{\circ}$ Brix. After heating, the sugar mass was allowed to rest for about 10 min, then gently stirred to initiate crystallisation. Stirring was then intensified to form coarse granules, which were crushed to break agglomerates and sieved through a 20-mesh sieve before packaging.

2.2.2. Analytical Methods

% Antioxidant Activity DPPH

Antioxidant activity was determined using the DPPH radical scavenging method with minor modifications [15]. One milliliter of sample solution was mixed with 2 mL of 25 μ M DPPH solution and incubated in the dark at room temperature for 30 min.

Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. A blank was prepared by replacing the sample with methanol. Antioxidant activity was expressed as the percentage of DPPH radical scavenging activity using the following equation:

$$\% \text{ Antioxidant Activity} = \frac{(\text{Abs blank} - \text{Abs sample})}{\text{Abs blank}} \times 100$$

Untargeted Liquid Chromatography–Mass Spectrometry (LC–MS/MS) Analysis

Untargeted metabolite profiling was performed using an Agilent Revident LC/Q-TOF system equipped with an electrospray ionisation (ESI) source operated in positive-ion mode. Chromatographic separation was achieved on a ZORBAX Eclipse Plus C18 RRHD column (2.1 × 50 mm, 1.8 µm) using a gradient elution of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The injection volume was 10 µL, and the total run time was 18 min. Mass spectra were acquired in full-scan mode over an *m/z* range of 50–1200, with MS/MS data obtained in auto MS/MS mode using collision-induced dissociation.

Data acquisition, processing, and feature extraction were carried out using Agilent MassHunter software based on accurate mass, retention time, and isotopic patterns. Compound annotation was performed by comparing precursor ions and MS/MS fragmentation patterns with spectral databases and literature. Owing to the untargeted approach and the absence of authentic standards, all compounds were considered putatively annotated. The analysis focused on relative differences in metabolite profiles rather than absolute compound identification.

Each palm sugar product (liquid, molded, and granulated) was prepared, and technical duplicate analyses were performed.. Each replicate was injected and analysed independently under identical LC–MS/MS conditions, and the resulting datasets were treated as independent observations for multivariate analysis. Compound identities were tentative and based on database matching without confirmation using authentic standards.

Since compound identification relied on database matching provided by the LC–MS software without confirmation using authentic standards, annotations were considered tentative and generally correspond to MSI Level 2.

3. RESULT AND DISCUSSION

The three products of palm sugar, including liquid palm sugar, molded palm sugar, and granulated palm sugar, are shown in Figure 1.

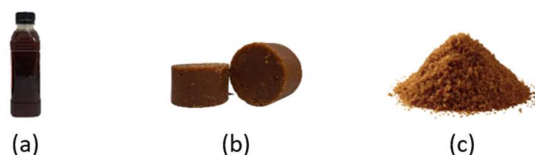


Figure 1. (a) liquid palm sugar, (b) molded palm sugar and (c) granulated palm sugar

3.1. Metabolite Profile of Palm Sugar Based on Untargeted LC–MS/MS Q-TOF Analysis

Untargeted LC–MS/MS analysis revealed differences in metabolite profiles among liquid, molded, and granulated palm

sugar, suggesting progressive chemical changes associated with increasing processing intensity. Representative LC–MS spectra of each product are presented in **Figure 2**.

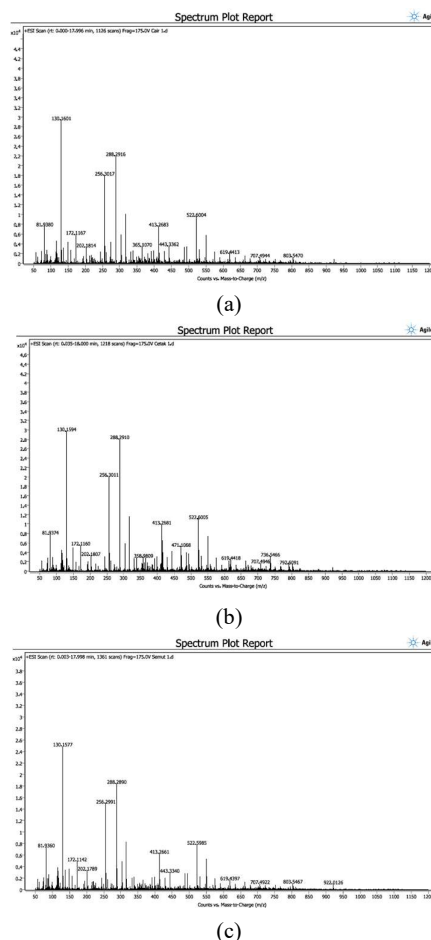


Figure 2. LC–MS/MS Q-TOF mass spectra of palm sugar samples showing ion intensity as a function of mass-to-charge ratio (*m/z*): (a) liquid palm sugar, (b) molded palm sugar, and (c) granulated palm sugar.

Liquid palm sugar showed the highest number of tentatively annotated features (95 compounds), followed by molded palm sugar (29 compounds) and granulated palm sugar (24 compounds). The decreasing number of detected compounds with increasing processing severity suggests a progressive reduction in metabolite complexity. This trend may be related to thermal degradation of heat-sensitive constituents, conversion of intermediate compounds, and increased matrix effects due to higher sugar concentration during evaporation. A summary of representative tentatively annotated compounds in each product is presented in **Table 1**.

All compounds listed in Table 1 were assigned at a putative annotation level based on accurate mass, retention behavior, and MS/MS fragmentation patterns. Therefore, these identifications should be interpreted as tentative rather than definitive. Nevertheless, the untargeted LC–MS results indicate systematic compositional shifts in palm sugar during processing, consistent with previous studies reporting that heating can substantially alter the chemical characteristics of sugar-rich food matrices depending on temperature and duration [16].

Table 1. Summary of annotated compounds in liquid, molded, and granulated palm sugar based on untargeted LC–MS analysis.

Compound	Formula	Compound Class	Products		
			LS	MS	GS
α -D-Glucose	C ₆ H ₁₂ O ₆	Monosaccharide	–	–	✓
Sucrose	C ₁₂ H ₂₂ O ₁₁	Disaccharide	✓	✓	✓
D-Maltose	C ₁₂ H ₂₂ O ₁₁	Disaccharide	✓	✓	–
Maltotriose	C ₁₈ H ₃₂ O ₁₆	Oligosaccharide	✓	✓	–
Isomaltulose	C ₁₂ H ₂₂ O ₁₁	Disaccharide	–	–	✓
Myo-Inositol	C ₆ H ₁₂ O ₆	Polyol	✓	✓	–
Maltol	C ₆ H ₆ O ₃	Oxygenated heterocyclic	–	✓	✓
2-Hydroxyhydroquinone	C ₆ H ₆ O ₃	Phenolic compound	–	–	✓

Notes: LS: Liquid Sugar; MS: Molded Sugar; GS: Granulated Sugar

Sucrose was tentatively detected in all palm sugar products, which is consistent with its role as the major carbohydrate constituent of palm sap-derived sweeteners [6]. In contrast, several oligosaccharide-related compounds varied among products. Features tentatively annotated as maltose and maltotriose were observed in liquid and molded palm sugar but were not detected in granulated palm sugar. This pattern may indicate that these compounds were present as intermediate products during partial carbohydrate transformation and subsequently decreased under more intensive heating conditions. Similar behaviour has been reported for certain sugars, which initially increase during early heating stages and decline after prolonged thermal treatment [17]. Monosaccharide-related changes were also observed. A feature tentatively annotated as α -D-glucose was detected only in granulated palm sugar, which may reflect further sucrose hydrolysis or carbohydrate breakdown during advanced processing. This interpretation is consistent with reports of increased reducing sugar content after longer heating due to sucrose hydrolysis [9]. Myo-inositol was tentatively observed in liquid and molded palm sugar but not in granulated palm sugar. Its absence in the most intensively processed sample may indicate reduced stability or lower detectability under severe heating conditions. Previous thermoanalytical studies have shown that myo-inositol can undergo a gradual thermal transformation under different heating conditions and physical states [18].

Several features associated with heat-induced reactions were more prominent in processed samples. A compound tentatively annotated as maltol was detected in molded and granulated palm sugar but not in liquid palm sugar. Maltol is widely recognised as a thermal reaction product generated during sugar degradation and caramelisation, and its presence may indicate increased thermal reactions at intermediate and advanced processing stages [19]. In addition, a feature tentatively annotated as 2-hydroxyhydroquinone was detected only in granulated palm sugar. This compound may be associated with the formation of low-molecular-weight phenolic or quinone-type structures generated through oxidation and thermal transformation of precursor compounds during intensive heating [20].

Overall, the LC–MS/MS results suggest that increasing processing intensity alters palm sugar composition by altering carbohydrate transformations, the loss of certain native constituents, and the formation of heat-derived compounds.

3.2. Principal Component Analysis (PCA) of Palm Sugar Samples

Principal component analysis (PCA) was applied as an unsupervised, exploratory multivariate approach to assess overall differences in metabolite profiles among liquid, moulded, and granulated palm sugar samples using untargeted LC–MS data. PCA reduces data dimensionality while retaining the major sources of variance, allowing visualisation of the sample distribution based on similarities in chemical composition [21]. The PCA biplot is presented on **Figure 3**.

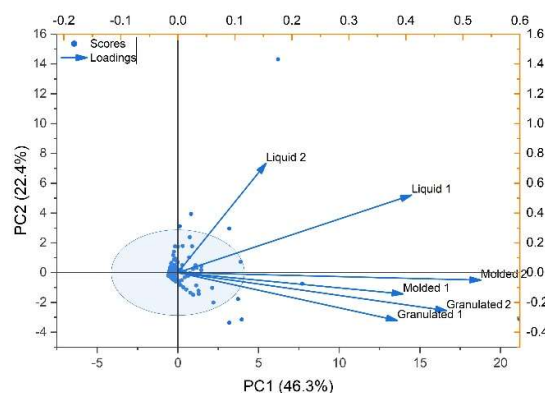


Figure 3. PCA biplot of palm sugar samples based on untargeted LC–MS data, sample distribution according to processing level (liquid, molded, and granulated palm sugar)

Figure 3 shows a tendency toward sample grouping by processing level. The first principal component (PC1) explained 46.3% of the total variance, while the second principal component (PC2) accounted for 22.4%, giving a cumulative explained variance of 68.7%. This indicates that the first two components captured a substantial proportion of the dataset's overall variability.

Liquid palm sugar samples were distributed mainly in the positive direction of PC2, whereas molded palm sugar samples occupied an intermediate position. Granulated palm sugar samples were separated from the other groups, suggesting progressive compositional changes associated with increasing thermal processing intensity.

Although the individual metabolites contributing most strongly to the observed separation were not specifically identified in this analysis, the PCA results provide supportive evidence that different processing levels were associated with distinct overall metabolite patterns in palm sugar.

3.3. Antioxidant Activity of Palm Sugar Samples

The DPPH radical scavenging activities of liquid, molded, and granulated palm sugar at a concentration of 1000 ppm are presented in **Figure 4**.

Figure 4 shows that granulated palm sugar exhibited the highest DPPH radical scavenging activity ($63.76 \pm 7.85\%$), followed by molded palm sugar ($55.93 \pm 8.25\%$) and liquid palm sugar ($39.23 \pm 5.83\%$). One-way ANOVA indicated that processing level significantly affected antioxidant activity ($F = 8.629$, $p = 0.017$). Tukey's post-hoc test showed that molded and granulated palm sugar had significantly higher antioxidant activity than liquid palm sugar ($p < 0.05$), while no significant difference was observed between molded and granulated palm

sugar. The statistical similarity between moulded and granulated palm sugar suggests that antioxidant-active compounds may have formed substantially during the moulded sugar stage, with further heating to granulated sugar resulting in only limited additional improvement.

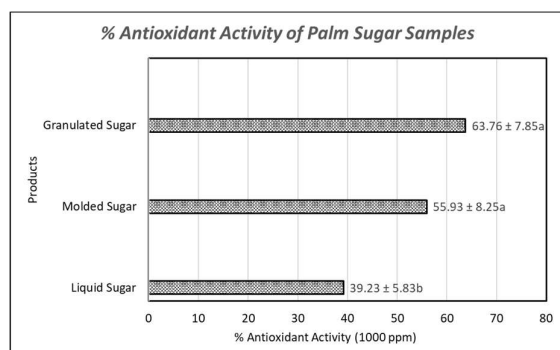


Figure 4. DPPH radical scavenging activity of liquid, molded, and granulated palm sugar at 1000 ppm concentration. Values are expressed as mean $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

The progressive increase in DPPH scavenging activity from liquid to molded and granulated palm sugar suggests that thermal processing was associated with the development of antioxidant-active compounds. The lower activity observed in liquid palm sugar may indicate that, at the early processing stage, radical scavenging properties were derived mainly from naturally occurring constituents originally present in palm sap. This interpretation is consistent with the LC–MS results (Table 1), in which fewer heat-derived compounds were tentatively detected in liquid palm sugar.

In contrast, the higher activity observed in moulded and granulated palm sugar may be due to chemical reactions that occur during heating, such as caramelisation and Maillard-type reactions. These thermal processes are known to generate compounds with hydrogen-donating or radical-quenching properties [22]. The tentative detection of maltol in molded and granulated palm sugar (Table 1) supports the occurrence of sugar degradation and formation of heat-induced reaction products during processing [23].

Antioxidant activity may also have been influenced by the formation of low-molecular-weight aromatic or phenolic compounds during advanced heating. A feature tentatively annotated as 2-hydroxyhydroquinone was detected only in granulated palm sugar, which may have contributed to its higher scavenging activity. Previous studies have reported that hydroquinone-related compounds can exhibit notable antioxidant behavior depending on structure and reaction environment [24].

Overall, these findings suggest that the antioxidant properties of palm sugar are associated not only with native compounds present in the raw sap, but also with processing-induced chemical transformations. Under the conditions studied, more intensive heating was associated with greater DPPH radical scavenging activity, particularly in granulated palm sugar. It should also be noted that excessive thermal treatment may promote the formation of undesirable compounds, such as advanced glycation end products (AGEs), and other heat-induced contaminants [25]. Therefore, optimising heating conditions is important to maximise functional benefits while minimising potential safety risks.

Although the number of detectable LC–MS features decreased with increasing processing severity, antioxidant activity increased. This apparent contrast may be explained by the degradation of multiple native minor constituents, together with the formation of fewer but more potent antioxidant-active compounds during heating, such as Maillard or caramelisation products. In addition, increased sugar concentration in highly processed samples may enhance matrix effects and reduce ionization efficiency of low-abundance metabolites, thereby lowering the number of detected LC–MS signals. Therefore, feature abundance does not necessarily correspond directly to functional antioxidant capacity.

4. CONCLUSION

Different processing levels during palm sugar production were associated with changes in metabolite composition and DPPH radical scavenging activity. Increasing thermal processing from liquid to moulded and granulated palm sugar was associated with shifts in detectable metabolite features, including reduced levels of several oligosaccharide-related compounds, the appearance of monosaccharide-related features, and tentative detection of heat-derived compounds such as maltol and phenolic-type derivatives.

These compositional changes were accompanied by higher DPPH radical scavenging activity, with granulated palm sugar showing the highest activity among the products evaluated. Principal component analysis further indicated differentiation among liquid, molded, and granulated palm sugar samples based on their overall metabolite profiles.

Overall, the results suggest that thermal processing modifies the chemical characteristics of palm sugar and may enhance its antioxidant-related properties under the conditions studied. In addition, untargeted LC–MS/MS was shown to be a useful approach for characterizing compositional changes during palm sugar processing and may support future product development and process optimization.

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