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# Investigating compositional and sulfite content variability in a range of sugar types using comparative analytical methodologies

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## INFO

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## ABSTRACT

Sugar production, with sugarcane as its primary raw material, positions Brazil as a global leader in crystal sugar output. During the sugarcane processing stages, sulfur derivatives, such as sulfite, are used as clarifying agents in white sugar production. The analysis of crystal sugar samples revealed their compliance with current legislation regarding parameters like conductivity ash, color, polarization, and insoluble mineral residue. However, the official ICUMSA methodology for sulfite analysis was tested against an alternative method based on the Monier-Williams reaction. The results obtained with the alternative method showed significantly higher sulfite values compared to the official method. Hot reflux distillation, used in the Monier-Williams method, demonstrated that the samples contained elevated sulfite levels, up to 6.9 (raw VHP), 25.8 (white type 1), and 13.9 (white type 2) times greater. The difference in results can be attributed to the fact that the hot reflux distillation process may promote the conversion of other sulfur derivatives present in the sample into sulfite, thus overestimating the values when compared to the official method. This finding showed that both methodologies are applicable, provided the analytical necessity is indicated, i.e., whether only sulfite levels or the totality of sulfur forms present in the sugar samples are required.

## RESUMO

**Palavras-chaves**
açúcar cristal
sulfito
ICUMSA
Monier-Williams
cana-de-açúcar
análise composicional

A produção de açúcar, tendo a cana-de-açúcar como principal matéria-prima, coloca o Brasil como um dos líderes mundiais na produção de açúcar cristal. Durante as etapas do processamento da cana, derivados de enxofre, como o sulfito, são utilizados como agentes de clarificação na produção de açúcar branco. A análise de amostras de açúcar cristal revelou que estas estão em conformidade com a legislação vigente em relação a parâmetros como cinzas condutimétricas, cor, polarização e resíduo mineral insolúvel. No entanto, a metodologia oficial da ICUMSA para análise de sulfito foi testada frente a uma metodologia alternativa, baseada na reação de Monier-Williams. Os resultados obtidos com o método alternativo apresentaram valores de sulfito expressivamente maiores em comparação com o método oficial. A destilação em refluxo a quente, utilizada no método Monier-Williams, demonstrou que as amostras continham teores mais elevados de sulfito, em até 6,9 (VHP bruto), 25,8 (branco tipo 1) e 13,9 (branco tipo 2) vezes maior. A diferença nos resultados pode ser atribuída ao fato de que o processo de destilação em refluxo a quente pode promover a conversão de outros derivados de enxofre presentes na amostra em sulfito, superestimando os valores encontrados quando comparados ao método oficial. Esse fato demonstrou que tanto uma quanto outra metodologia são aplicáveis desde se indique a necessidade analítica, ou seja, se apenas teores de sulfito ou a totalidade das formas de enxofre presentes nas amostras de açúcar.

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## INTRODUCTION

Among the most prominent products derived from sugarcane, sugar stands out as a key commodity. Brazil ranks among the world's leading producers and exporters, and according to The Food and Agriculture Organization (Miranda & Fonseca, 2020), global sugar trade is projected to expand over the next decade, driven by rising demand. The bulk of this additional production will come from major players such as Brazil, India, and China (Solomon, 2016; Medina & Costa, 2023; Almeida & Colombo, 2023). To meet stringent international standards, cutting-edge technologies have been integrated into sugar production, storage, distribution, and packaging, optimizing efficiency and ensuring superior quality (Alam *et al.*, 2025; George, 2024).

One of the critical stages in crystal sugar production is clarification and purification (Figure 1), executed through the sulfo-defecation method (Di-Tanno *et al.*, 2021; Ogando *et al.*, 2021; Sartori *et al.*, 2017). This process leverages the action of sulfite to decolorize sugarcane pigments, ensuring a cleaner and more refined final product (Wang *et al.*, 2023). Additionally, the reaction with sulfur dioxide ($SO_2$) plays a pivotal role by blocking the carbonyl functional group, thereby preventing the formation of colored oligosaccharides and enhancing the sugar's purity and visual appeal (Rein, 2007).

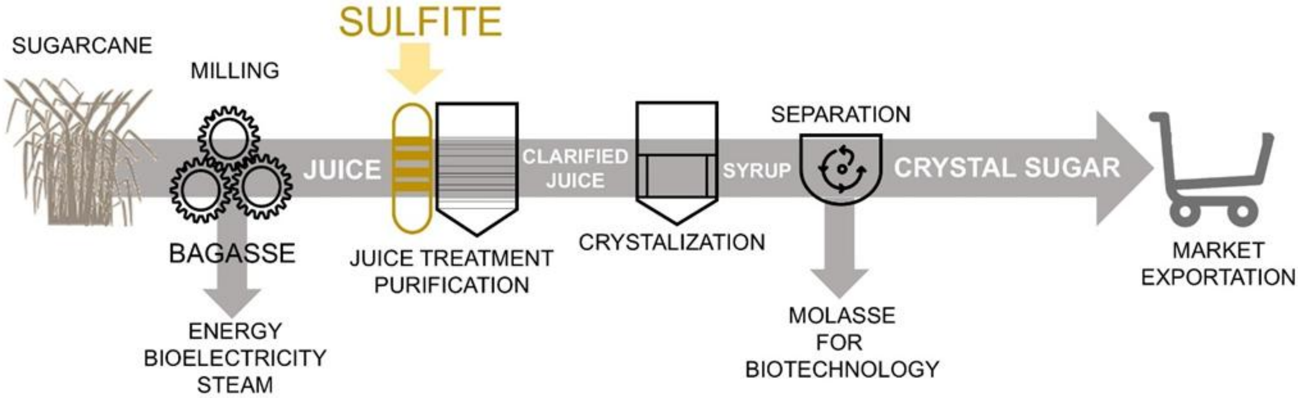

Figure 1 - Simplified schematic of the unit operations involved in crystal sugar production in sugarcane mills. Highlighted in yellow: the sulfitation unit operation, representing the introduction of sulfur dioxide in the white sugar manufacturing process

The amount of sulfur consumed in sugar clarification depends on several factors, including the purification method adopted, the target color specification, the desired final acidity, and the quantity of lime added during the subsequent neutralization stage (liming) (Eggleston *et al.*, 2017). When juice sulfitation is employed, sulfur consumption ranges from 250 to 500 mg/kg of sugarcane. If both the juice and syrup undergo sulfitation, this figure can rise to as much as 900 mg/kg of cane (Rein, 2007). The application of sulfur in oxygen-deprived environments can facilitate the formation of total reduced sulfur compounds (TRS), such as hydrogen sulfide ($H_2S$), methyl mercaptan ($CH_3SH$), dimethyl sulfide ($(CH_3)_2S$), and dimethyl disulfide ($(CH_3)_2S_2$) (Postgate, 1979). Some of these compounds are linked to adverse health effects, including headaches, nausea, insomnia, and irritation, with potential neurological damage and, in extreme cases, fatal outcomes (Beauchamp *et al.*, 1984; Khalaf *et al.*, 2024). While recognized as safe for most consumers, sulfites also pose a risk to sensitive individuals, particularly those with asthma, potentially triggering respiratory distress, gastrointestinal irritation, and headaches (Gupta & Basavaraj, 2021; Witkowski *et al.*, 2022).

Derived from sugarcane in tropical region, crystal sugar is a cornerstone of the food industry, playing a pivotal role in the production of confections, pastries, cookies, beverages, and preserves. Its versatility makes it an indispensable ingredient in baking and the large-scale manufacturing of industrialized drinks, ensuring texture, consistency, and flavor in a vast array of culinary applications (Chandran *et al.*, 2024). Excessive sugar consumption is undeniably linked to a range of chronic diseases, including type 2 diabetes, obesity, and cardiovascular disorders (Malik & Hu, 2022). However, beyond its metabolic impact, concerns arise regarding the presence of chemical additives or residual byproducts from the sugarcane treatment process, necessitating stringent oversight by regulatory bodies to ensure food safety (Wani *et al.*, 2023; Dordevic *et al.*, 2023). Sulfites are among the most

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widely used preservatives in the food and beverage industry, effectively preventing spoilage and microbial growth (Novais *et al.*, 2022). Commonly found in wine, dried fruits, juices, and processed foods, these compounds are also present in certain types of sugar. Regulatory frameworks enforce stringent guidelines, not only mandating explicit labeling of sulfite content to enhance consumer awareness and well-being but also emphasizing the necessity of precise quantification in food products (Jadhav *et al.*, 2025; Chang *et al.*, 2023). This applies not only to sugars intended for direct consumption but also to those used as key ingredients in complex food formulations, ensuring compliance with safety standards and minimizing potential health risks.

Considering reports of residual concentrations of TRS compounds in white crystal sugar samples — where the likelihood of occurrence is higher — and in raw sugars, where the probability is lower, as well as the association of these compounds with potential adverse health effects, this study aimed to monitor and quantify sulfite concentrations in sugar samples sourced from three different production units of white crystal sugar and VHP (Very High Polarization) raw sugar.

## MATERIAL AND METHODS

### Sugar samples and their collection

Samples of white sugar type 1, white sugar type 2, and raw sugar (VHP) were collected from three sugar mills in the central region of the state of São Paulo, Brazil. To further expand the scope of our investigation, samples of white crystal sugar were also procured from retail establishments within the municipality of Piracicaba, São Paulo State. This inclusion was prompted by the observed considerable variation in sulfite levels among the samples collected directly from the sugar mills. Consequently, a critical concern arose regarding the potential presence of elevated sulfite concentrations in sugar products readily available to consumers in a representative urban center. The sugar sampling at the mills followed a rigorous randomization protocol and was conducted directly from the conveyor system, i.e., samples were systematically gathered from the conveyor belt immediately following the sugar dryer, at the warehouse entry point, or, during the bagging process for shipment. Each sample, comprising approximately 1 to 2 kg of sugar for each type, was meticulously stored in polypropylene plastic bags. These bags were then expeditiously transported to the laboratory, where they underwent hermetic sealing using a vacuum sealer to ensure sample integrity. Conversely, samples of commercially available white crystal sugar from Piracicaba's local market were acquired in 1 or 2 kg packages, randomly selected from various grocery stores. This assortment consisted of approximately 10 distinct samples of type 1 white crystal sugar, providing a comprehensive representation of consumer-grade products.

Figure 2 - Principal regional location of tested sugar sample collection, with samples obtained from both direct sugarcane mill and local regional markets

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## Compositional characterization of sugar samples

The white crystal sugar and raw sugar samples were analyzed according to their respective official methodologies described in the Handbook of Methods of The International Commission for Uniform Methods of Sugar Analysis Ltd. (ICUMSA, 2017). All analyses were performed in analytical triplicate, using three collections of each type of sugar. The values were described as the mean of these repetitions, followed by their standard deviations.

The polarization, or apparent sucrose content, of the white crystal sugar was determined utilizing the ICUMSA GS2/3-1 methodology. Initially, a precise 26 g aliquot of each sugar sample was meticulously solubilized in 100 mL of ultrapure distilled water. Subsequently, the obtained solution was subjected to filtration and analyzed within a crystal tube, featuring a 200 mm optical path, employing an ADS 420 model polarimeter/saccharimeter, manufactured by Bellingham + Stanley Ltd. (Kent, United Kingdom). Conversely, the analysis of VHP raw sugar necessitated the application of the ICUMSA GS1/2/3-2 methodology. This procedure maintained the identical solubilization ratio but incorporated the clarifying agent lead subacetate to effectively eliminate turbidity-inducing particulates. Following this clarification step, the solution underwent filtration and subsequent polarimetric analysis, adhering to the protocol previously described.

For the conductimetric ash analysis of white crystal sugar, a solution was prepared at a concentration of 26 g of sample per 100 g of ultrapure distilled water, adhering to the ICUMSA GS2/3/9-17 methodology. The resultant solution was then subjected to direct conductivity measurement utilizing a Tec-4MP model conductivity meter (Tecnal, Brazil). In contrast, the determination of conductimetric ash in VHP raw sugar necessitated the preparation of a solution at a concentration of 5 g of sample per 100 g of ultrapure distilled water, in accordance with the ICUMSA GS1/3/4/7/8-13 method. This solution was subsequently analyzed using the same conductivity measurement technique.

The ICUMSA color determination of white crystal sugar was based on the ICUMSA GS2/3-10 methodology. Sugar samples were weighed and solubilized in distilled water at a 1:1 ratio (25 g of sample in 25 g of distilled water), and then vacuum-filtered through cellulose acetate membranes with a 0.45 µm pore diameter. The filtered solution was subjected to reading at 420 nm in a UV-Mini 1240 model spectrophotometer (Shimadzu, Japan), using a 1 cm path length quartz cuvette. The ICUMSA color value was calculated according to Equation 1.

Where b is the optical path length of the cell (cm; in this case: 200 mm); and c is the sucrose concentration in g/mL in the sugar solution as a function of Brix (soluble solids content previously determined by direct reading with a digital refractometer) at 20°C (temperature automatically corrected by the equipment).

For the ICUMSA color analysis of VHP sugar, the ICUMSA GS1/3-7 methodology was used. The samples were weighed and solubilized in water at a ratio of 30 g of sample to 70 g of distilled water. Subsequently, the pH of the solution was adjusted to 7.0±0.1, using a 0.1 mol/L solution of hydrochloric acid or sodium hydroxide. The samples were vacuum-filtered through cellulose acetate membranes with a 0.45 µm pore diameter, and finally, the solution was subjected to absorbance reading at 420 nm in a spectrophotometer, as previously described. The ICUMSA color values of the VHP sugar samples were also calculated according to Equation 1.

The analyses of insoluble mineral residues for crystal sugar and VHP sugar were performed according to ICUMSA GS2/3/9-19. Ten g of sample were weighed and diluted in 20 mL of distilled water. Subsequently, this solution was vacuum-filtered through a cellulose acetate filtration membrane with a 0.45 µm pore diameter, and then the filtration membranes with the filtered residue were placed in an oven at 60°C for 1 h. Next, the samples were kept in a desiccator with a desiccant agent (silica gel) for a period of 30 min until temperature equilibrium with the environment. Finally, each membrane containing the mineral residue was weighed on an analytical balance with a precision of 0.001 g. Thus, the insoluble mineral residue content, expressed in mg/kg, was determined using Equation 2:

Where m1 is the mass in grams of the membrane; m2 is the mass in grams of the membrane after filtration; and m0 is the mass of the collected sample.

## Determination of sulfite by the ICUMSA official method and by the reflux distillation method

Sulfite in white and VHP raw sugar was quantified via ICUMSA GS2/1/7/9-33. Eight sucrose solutions (10 g sucrose/60 mL water, 18.2 mΩ/cm resistivity) were prepared in 100mL flasks, with 0-15mL sodium sulfite (0.0002 mol/L) and 4 mL NaOH (0.1 mol/L) added. 10 mL aliquots were transferred to tubes, with 2 mL decolorized fuchsin (or *p*-rosaniline) and 2 mL 0.2% formaldehyde.

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Absorbance was read at 560 nm (UV-Mini 1240, 1 cm quartz cuvette). Samples were compared to a calibration curve, yielding sulfite in mg/kg, according to equation below. ICUMSA GS2/1/7/9-33's fuchsin color instability causes spectrophotometric deviations and sulfite quantification inaccuracies.

To address ICUMSA's color instability, we developed a new sulfite determination method for white crystal sugar using a nitrogen distillation unit. Sulfite was quantified by comparing samples to a blank or a standard curve, according to Monier-Williams reactions. Both used HCl (25%), iodine (0.05 mol/L), $Na_2S_2O_3$ (0.01 mol/L), and $H_2SO_4$ (0.5 mol/L). Iodine concentration was titrated with $Na_2S_2O_3$. A blank was distilled: nitrogen distillator preheated; 100 mL water + 100 mL HCl (25%) in sample vessel; 30 mL + 50 mL water in receivers 1 & 2; 4 mL & 1 mL iodine (0.05 mol/L) added, respectively. Distillation (6 min, 100% steam). Combined receiver volumes were acidified with 2 mL $H_2SO_4$ and titrated with 0.01 mol/L $Na_2S_2O_3$. Subsequently, the sugar samples were distilled and titrated following the same procedure as the blank, except that 100 g of the sample was used instead of 100 mL of water in the 500 mL sample vessel. The sulfite concentration in the samples was then determined using Equations 3 through 8, utilizing the titration results from both the blank and the samples.

1. $c_{eff}(I_2) = \frac{V_{eff}(Na_2S_2O_3) \times C(Na_2S_2O_3)}{V_{eff;sample}(I_2) \times z}$
2. $n_{blank}(SO_2) = V_{eff;blank}(I_2) \times c_{eff}(I_2) - \frac{V_{blank}(Na_2S_2O_3) \times c(Na_2S_2O_3)}{z}$
3. $n_{sample}(SO_2) = V_{eff;sample}(I_2) \times c_{eff}(I_2) - \frac{V_{sample}(Na_2S_2O_3) \times c(Na_2S_2O_3)}{z}$
4. $n_{eff}(SO_2) = n_{sample}(SO_2) - n_{blank}SO_2$
5. $n_{eff}(SO_2) = n_{sample}(SO_2) - n_{blank}SO_2$
6. $SO_2(mg/L) = \frac{SO_2(mg) \times 1000}{V_{sample}}$

Where:

| | | |
|---|---|---|
| $c_{eff}(I_2)$ | Effective concentration of iodine solution | mol / L |
| $V_{eff;}(Na_2S_2O_3)$ | Consumption of $Na_2S_2O_3$ solution for determination of $C_{eff}(I_2)$ | L |
| $c(Na_2S_2O_3)$ | Concentration of sodium thiosulfate solution | mol / L |
| $V_{eff;blank}(I_2)$ | Volume of iodine solution in blank determination | L |
| z | Reaction valence | - |
| $n_{sample}(SO_2)$ | Amount of $SO_2$ in sample determination | mol |
| $V_{eff;sample}(I_2)$ | Consumption of iodine solution for sample determination | L |
| $V_{sample}(Na_2S_2O_3)$ | Consumption of $Na_2S_2O_3$ for sample determination | L |
| $N_{blank}(SO_2)$ | Amount of $SO_2$ for blank determination | mol |
| $V_{blank}(Na_2S_2O_3)$ | Consumption of $Na_2S_2O_3$ solution for blank determination | L |
| $n_{eff}(SO_2)$ | Amount of $SO_2$ actually in the sample | mol |
| $SO_2(mg)$ | Mass of $SO_2$ in the sample | mg |
| $M(SO_2)$ | Molar mass of $SO_2$ | g/mol |
| $SO_2(mg/L)$ | Concentration of $SO_2$ in the sample | mg/L |
| $V_{sample}$ | Volume of the sample | L |

The second part of the process involved creating a standard curve using the equipment. The aim was to eliminate the need to run a blank sample, thus streamlining the analysis process. Another objective was to assess the method's accuracy using the blank. To achieve this, five solutions of analytical grade sucrose with varying concentrations of sulfite (using 100 g of sucrose) were prepared. Next, 100 mL of the curve solutions and 100 mL of hydrochloric acid (25%, v/v) were added to a 500 mL sample container from the distillation apparatus. After distillation, titration was performed with iodine solutions on the receivers, and the amounts of sodium thiosulfate consumed were recorded. Subsequently, the samples were distilled and titrated, and the amounts of sodium thiosulfate consumed were correlated with the concentration based on the previously prepared standard curve.

### *Statistical analyses*

The sugar types were compared based on the mean values of compositional analyses (polarization, conductivity ash, ICUMSA color, and insoluble mineral residues) and sulfite content through Analysis of Variance (ANOVA) and

Tukey's mean comparison test. Efforts were made to correlate the compositional characteristics with sulfite content using Pearson's correlation. Finally, sulfite content measured via the reflux distillation method was compared with the ICUMSA GS2/1/7/9-33 methodology. All analyses were conducted using RStudio 1.2.5033 statistical software. Chemometric analyses were employed to assess the similarity between samples. Principal Component Analysis (PCA) and hierarchical clustering were performed using MetaboAnalyst v. 6.0 (free online package; Pang *et al.*, 2021).

## RESULTS AND DISCUSSION

### Compositional Analysis of Sugar Samples

A total of 25 sugar samples from different types, collected from both sugar mills and local markets, were analyzed, and the results are presented in Table 1. The compositional analyses, with the exception of insoluble residues, indicated that the samples met the specifications outlined in Decree No. 55871 of March 26, 1965, and Normative Instruction No. 47 of August 30, 2018 (BRASIL, 1965; MAPA, 2018). The VHP sugar exhibited the lowest apparent sucrose concentration (ºZ or polarization) and the highest values for conductivity ash (%) and ICUMSA color (UI). All of these values differed significantly ($p$<0.05) from those of the white sugars, type 1 and type 2. In contrast, the white sugars differed only in ICUMSA color (with type 1 showing the lowest value), while no significant differences were observed between them in polarization and conductivity ash (%).

Table - Compositional characterization of white (type 1 and 2) and raw (VHP) sugars.

| Parameters | Sugar type | Average | Standard deviation |
|---|---|---|---|
| Polarization (°Z) | Raw VHP | 96.7$^{b}$ | 0.71 |
| | White type 1 | 99.1$^{a}$ | 0.30 |
| | White type 2 | 98.5$^{a}$ | 0.03 |
| Conductimetric ashes (%) | Raw VHP | 0.12$^{a}$ | $5\times10^{-6}$ |
| | White type 1 | 0.05$^{b}$ | $3\times10^{-5}$ |
| | White type 2 | 0.05$^{b}$ | $5\times10^{-6}$ |
| ICUMSA color (UI) | Raw VHP | 1,321.5$^{a}$ | 3.1 |
| | White type 1 | 163.2$^{c}$ | 0.92 |
| | White type 2 | 288.4$^{b}$ | 9.5 |
| Insoluble residues (mg/kg) | Raw VHP | 1,755$^{b}$ | 219.2 |
| | White type 1 | 2,775$^{a}$ | 49.5 |
| | White type 2 | 450$^{c}$ | 141.42 |

For each analysis, Analysis of Variance (ANOVA) and Tukey's mean separation test were performed. Distinct letters following the means indicate a significant difference at the 5% level between the samples

These results reflect the intended use and commercialization of the different types of sugar. VHP sugar will be reprocessed before consumption; therefore, at the end of the process, it is not suitable for ingestion. Its primary destination is export, and there are no concerns regarding ICUMSA color, with the minimum polarization being more flexible. On the other hand, white crystal sugar is generally directed to the domestic market for direct consumption or used as an additive in food processing, such as in sweets, soft drinks, cookies, and so on. Thus, sweetness and appearance attributes are crucial in determining the product’s value (Aguiar *et al.*, 2015; Dias *et al.*, 2015; Duarte *et al.*, 2019). Therefore, a significant difference in all compositional attributes was expected. The main factor differentiating the sugar characteristics lies in the treatment and storage conditions. During the production of VHP sugar, no sulfite is added to reduce ICUMSA color. Additionally, the product is typically stored in bulk without strict microbiological or physical control measures (Egorova et al., 2025; Campiol et al., 2019). These storage conditions may lead to greater exposure to microbial growth and physical degradation, such as color darkening caused by Maillard reactions and oxidation. Several studies have shown that uncontrolled environmental

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conditions during storage can accelerate non-enzymatic browning and contribute to the formation of impurities in sugar products. In contrast, white sugar production involves sulfitation—usually with sulfur dioxide or bisulfite—in concentrations proportional to the desired whiteness. This process significantly reduces ICUMSA color and helps stabilize the product during storage. Gorodetsky et al. (2022) reported that sulfitation decreased color reversion by up to 18% compared to untreated samples, making it a crucial step in maintaining color stability and microbiological safety.

Moreover, white sugar is stored under stricter conditions to prevent caking, yellowing, and the appearance of black spots. Among the tests conducted, only the analysis of insoluble residues showed inconsistent results. A plausible explanation is methodological variability at different stages of the analysis. Minor inconsistencies in sample preparation, equipment calibration, or operator technique may have contributed to the observed fluctuation. Chemometric analysis based on sugar characteristics revealed clear discrimination between the different types of crystalline sugar. Principal Component Analysis (PCA), a widely used statistical method in food quality studies, was applied and successfully clustered the three sugar types into distinct groups. Similar applications of PCA combined with FTIR or NIR spectroscopy have been reported in recent literature for classifying sugar mixtures and monitoring processing stages (Silva et al., 2024). Despite this clear clustering, some data dispersion was observed within White type 2 samples, possibly reflecting heterogeneity in processing or raw material origin. PC1 accounted for 90.1% of the total variance, which is relatively high but still suggests the presence of additional sources of variation not fully captured by the first component. This residual variability is common in spectral datasets, especially when dealing with complex food matrices. Studies involving sugar fluorescence and advanced chemometric modeling, such as PARAFAC or PCA, have documented similar patterns of intra-cluster variation (Baunsgaard et al., 2000).

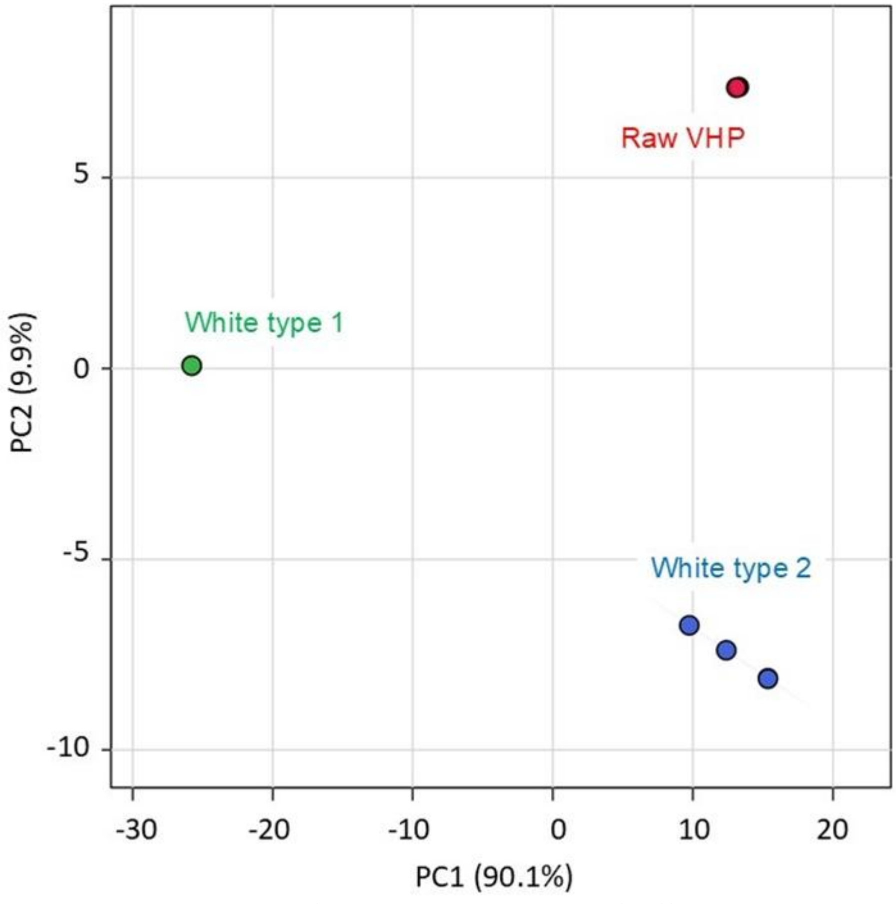

Figure 3 - Principal component analysis of the compositional data of different sugar types

Cluster and hierarchical analysis are two widely used methods for examining statistical data,

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particularly in similarity studies and in identifying key variables (Jaeger & Banks, 2023). Based on the data from the sugar characteristics, both clusters and hierarchical analysis indicate a strong discrimination between the different sugar types. However, a considerable variability within each group was also observed, suggesting intrinsic differences among samples within the same classification.

The VIP score analysis (Figure 4) revealed statistically significant differences among the measured variables — polarization, color, ashes, and mineral residue — with polarization standing out as the most influential factor, particularly in white sugar type 2, followed by raw VHP sugar. The color played a crucial role in distinguishing raw VHP sugar samples, whereas for white sugar type 1, the most significant variable was insoluble mineral residue. In contrast, conductivity ash emerged as the key variable for white sugar type 2. These findings are further supported by Boxplot analysis (Figure 5), despite the high variability observed in the conductivity ash values for white sugar type 2, as evidenced by the broad range of its boxplot.

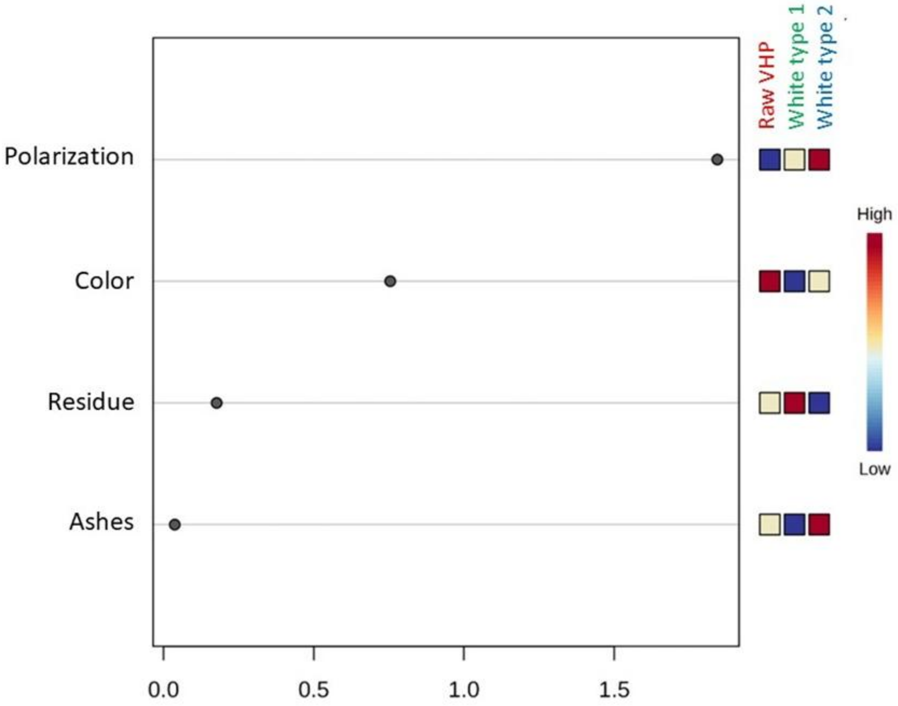

Figure 4 - Variable Importance in Projection (VIP) scores for the analysis of different types of crystal sugar

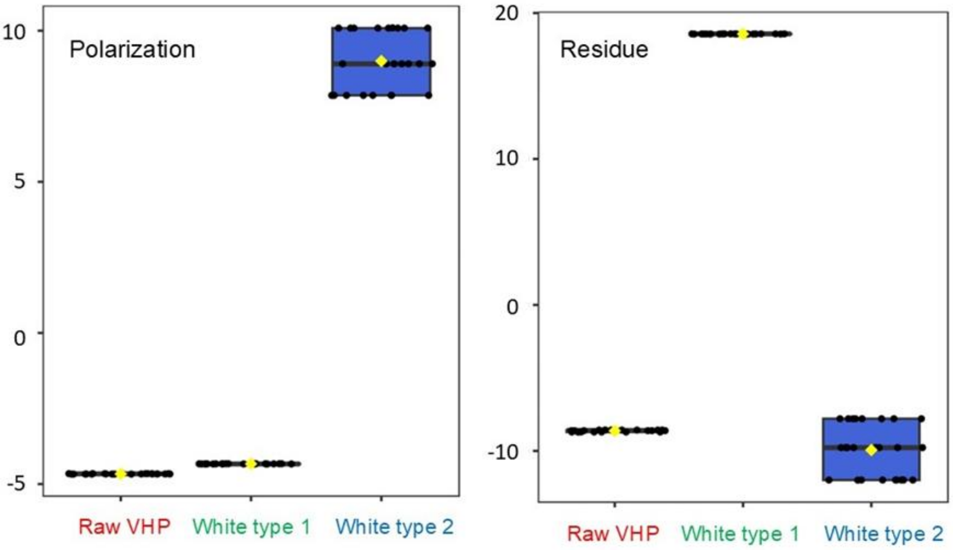

Figure 5 - Comparative analysis of compositional feature distribution in crystal sugars using Boxplots

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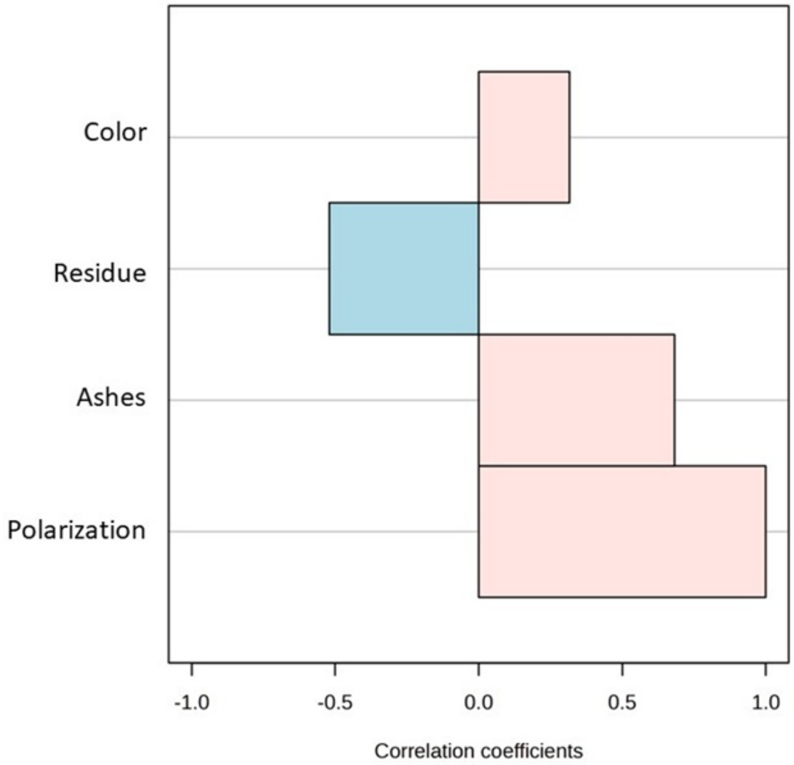

Figure 6 - Identification of distinctive patterns in crystal sugars by Pattern Hunter analysis

Among all the variables analyzed, insoluble mineral residue emerged as the most significant factor contributing to the variability and differentiation of sugar types (Figure 6), a finding consistent with previous studies that highlight residue as a key determinant of sugar quality and processing performance (Awulachew *et al.*, 2025). The horizontal bar graph illustrates the correlation between four variables (Color, Residue, Ashes, and Polarization) and the polarization factor, through correlation coefficients. Polarization demonstrates the strongest positive correlation, indicating a significant direct relationship with the factor, corroborating results from sugar storage and purity studies that show polarization declines in the presence of increasing impurities. The study by Ramadan *et al.* (2022) shows that polarization directly influences the physicochemical properties of sugar, reflecting its purity and quality. Meanwhile, Color and Ashes also show moderate positive correlations, suggesting that higher values in these variables tend to accompany higher values of the factor. This trend aligns with findings that link increased ash and color content with darker and more mineral-rich sugar samples. In contrast, residue exhibits a moderate negative correlation, indicating an inverse relationship where higher residue values are associated with lower values of the factor—again supported by strong negative associations between insoluble matter and sugar quality attributes. The magnitude of the bars reflects the strength of the correlation, and the direction (right or left of zero) indicates the positive or negative nature of the relationship. The hierarchical analyses (Figures 7 and 8) revealed that, despite a high degree of variability in variable patterns within each sugar group, there is also strong difference between sugar types. The dendrogram and heatmap analyses identified color as the primary differentiating factor among sugar types, highlighting the minimal intensity of white sugar type 1 relative to white sugar type 2 and raw VHP sugar. Interestingly, the color profiles of white sugar type 2 and raw VHP sugar appear more closely related, reducing their statistical differences. However, this does not imply that these sugar types are similar — quite the opposite. Both dendrogram and heatmap analyses provide statistical evidence that these sugar types are distinct from one another, reinforcing their unique compositional characteristics.

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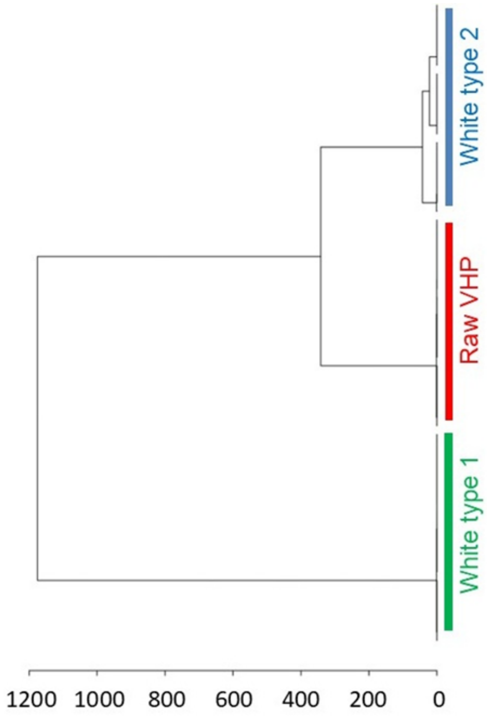

Figure 7 - Dendrogram showing relationships between different sugar types

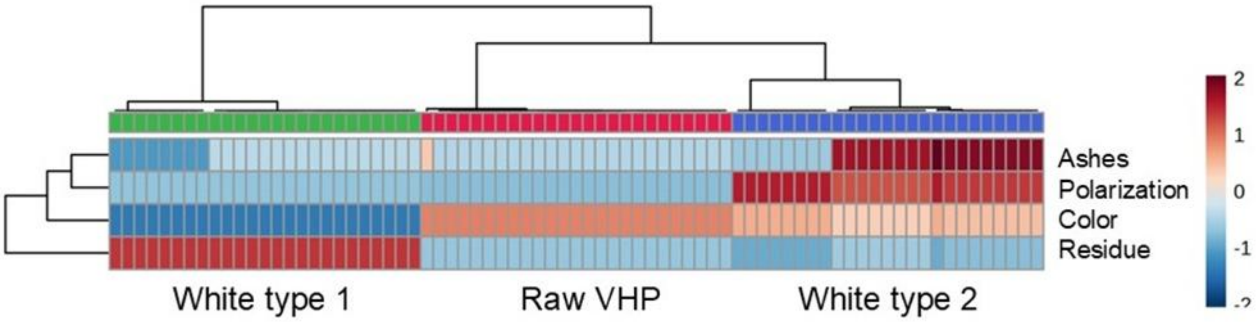

Figure 8 - Heatmap of sugar samples based on compositional analysis

### *Presence and quantification of sulfite levels in sugar samples*

$SO_2$ concentrations for white sugar type 1, white sugar type 2, and raw VHP sugar were 0.93 mg/kg, 1.92 mg/kg, and 2.52 mg/kg, respectively. The Tukey test ($p<0.05$) confirmed that all three means were significantly different, with VHP sugar exhibiting the highest $SO_2$ concentration, followed by white sugar type 2, and finally white sugar type 1 with the lowest value. An unexpected trend was observed in the $SO_2$ concentration hierarchy. As previously mentioned, in white sugar type 1 production, sulfitation is applied more intensively to achieve greater color removal; however, regulatory standards impose strict limits on residual sulfite levels, particularly for sugars intended for direct consumption. In contrast, VHP sugar production involves only minimal sulfur application, primarily to enhance juice fluidity in decanters. Nevertheless, no regulatory limits exist for residual sulfite levels in raw sugars like VHP, as these sugars undergo further processing in their destination countries after export, ensuring compliance with local standards before reaching consumers. The maximum allowable sulfur residue limit for white crystal sugar is 10 mg/kg, while VHP sugar has no regulatory limit, meaning that all three sugar types analyzed comply with the specifications outlined in Normative Instruction No. 47 of August 30, 2018 (BRASIL, 1965; MAPA, 2018).

The sulfite content analysis using the reflux distillation method was also conducted, and the results are discussed below. The first step involved determining the effective iodine concentration, ceff($I_2$). After three titration repetitions, the

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effective concentration was found to be 0.0492 mol/L. Since the same reagents were used for all three sugar samples, only one blank distillation was performed, consuming 4.87 mL of $Na_2S_2O_3$ solution, which corresponded to $2\times10^{-6}$ mol $SO_2$ in the blank. The sugar samples were distilled, and the iodine solution was titrated to determine the sulfur content. The average $Na_2S_2O_3$ consumption was 4.33 mL for VHP sugar, 4.12 mL for white sugar type 1, and 4.04 mL for white sugar type 2. The calculated moles of $SO_2$ were $2.9\times10^{-5}$ mol in VHP sugar, $3.95\times10^{-5}$ mol in white sugar type 1, and $4.38\times10^{-5}$ mol in white sugar type 2. After subtracting the total $SO_2$ content in the blank, the effective $SO_2$ concentrations in the samples are presented in Table 2.

Table 2 - Quantification of $SO_2$ in VHP sugar, white sugar type 1, and white sugar type 2: molar amount, mass (mg), and concentration (mg/kg)

| **Samples** | **$n_{eff}SO_2$ (mol)** | **$SO_2$ mass (mg)** | **$SO_2$ concentration (mg/kg)** |
|---|---|---|---|
| Raw VHP | $2.70\times10^{-5}$ | 1.73 | 17.30 $^{b}$ |
| White type 1 | $3.75\times10^{-5}$ | 2.40 | 24.02$^{ab}$ |
| White type 2 | $4.18\times10^{-5}$ | 2.68 | 26.75 $^{a}$ |

Analysis of variance (ANOVA) and Tukey's test for differentiation of means were performed according to the $SO_2$ concentration. Different letters after the means indicate that there is a significant difference of 5% between the samples.

Using a commercial reflux distillation system, the residual $SO_2$ levels in VHP sugar were found to be the lowest among the analyzed samples. However, the $SO_2$ content in white sugar type 2 did not differ significantly from that in white sugar type 1 at a 5% significance level. One possible explanation for this is that during the decantation stage of white sugar type 1, a greater amount of sulfur was removed from the juice. In other words, while a higher $SO_2$ concentration was applied to produce whiter sugar, the process also proved to be more efficient in removing the applied sulfur. This outcome is likely due to enhanced decantation efficiency. Lime treatment is performed to adjust the pH and promote the formation of calcium and sulfur flocs. The juice then undergoes decantation, where colloidal aggregates form between the calcium-sulfur flocs and impurities. During this process, polymers are introduced to facilitate flocculation, leading to sedimentation and flotation. By the end of the process, a gravitational separation occurs, yielding a heterogeneous three-phase mixture: (1) the top layer (less dense colloids - floated), (2) the middle layer (clarified juice), and (3) the bottom layer (denser colloids - settled). As a result, $SO_2$-bound coagulates are directed into the decantation sludge rather than proceeding to the evaporation stage (Rein, 2007).

Pearson’s correlation analysis revealed a strong positive correlation (r = 0.86) between $SO_2$ content (determined via reflux distillation) and polarization, while conductivity ash (r = -0.96) and ICUMSA color (r = -0.93) exhibited strong negative correlations. This indicates that higher residual $SO_2$ concentrations were associated with lower ICUMSA color values and fewer mineral residues, as well as higher sucrose content, suggesting that more impurities were removed, resulting in a purer sugar product.

However, the measured $SO_2$ concentrations exceeded the regulatory limits, with white sugars surpassing the 10 mg/kg threshold established by legislation (BRASIL, 1965; MAPA, 2018). These findings prompted the development of a simplified methodology using the distillation system, achieved through the creation of a standard curve with sucrose and sulfite solutions.

The determination of sulfite in sugars can be performed using different methodologies, with the official *p*-rosaniline method (ICUMSA GS2/1/7/9-33) widely used for regulatory analyses, while reflux distillation emerges as an investigative alternative. The official method is based on the reaction between free sulfite and the *p*-rosaniline reagent, forming a pink complex whose color intensity is measured spectrophotometrically.

$$SO_2 + H_2O \rightarrow HSO_3^- + H^+$$

$$HSO_3^- + CH_2O \rightarrow CH_2(OH)SO_3^-$$

$$CH_2(OH)SO_3^- + p - rosaniline \rightarrow color\ complex\ (purple\ or\ pink)$$

Figure 9. Reactions involved in the official methodology for sulfite determination by colorimetry using p-rosaniline.

This procedure offers high sensitivity, capable of detecting low $SO_2$ concentrations, but it may suffer from interference caused by colored compounds present in sugar, such as impurities and natural pigments. On the other hand, the reflux distillation method involves releasing $SO_2$ through acid distillation, followed by its quantification via titration with iodine and sodium thiosulfate. This method is less susceptible to interference since the $SO_2$ is isolated before titration, ensuring greater selectivity.

However, its sensitivity may be slightly lower than that of the spectrophotometric method, and it requires strict control of experimental conditions to ensure reproducibility. In terms of precision, the *p*-rosaniline method (fuchsin) is standardized and widely used for comparative analyses and quality control, making proper calibration curve preparation essential to avoid variations in results. Meanwhile, the reflux distillation method demonstrates good precision, but its accuracy depends on meticulous temperature control and manual titration, factors that may introduce minor variations. Considering time and ease of execution, the *p*-rosaniline method stands out for being faster, delivering results within minutes. However, it involves toxic reagents and requires special handling precautions. In contrast, reflux distillation takes more time as it includes distillation, collection, and titration steps, in addition to requiring specific equipment, such as a reflux distiller system, which may limit its applicability in laboratories without such infrastructure.

From a regulatory perspective, the *p*-rosaniline method is widely accepted by oversight agencies and is mandatory for certifying sugars intended for international trade. Meanwhile, reflux distillation, though not yet officially recognized, can serve as a complementary tool for validating results and conducting more detailed investigations into the behavior of residual $SO_2$ in different sugar types. Selecting the most suitable method depends on the purpose of the analysis. The *p*-rosaniline method is ideal for rapid analyses and official certification, whereas reflux distillation can provide a more precise estimate of the actual residual $SO_2$ content, isolating it from potential interfering substances. Therefore, the complementary use of both methods can be a strategic approach, combining the speed and standardization of the official method with the selectivity and reliability of reflux distillation for in-depth studies on $SO_2$ removal during industrial sugar processing.

### *Analysis of the difference between the sulfite concentrations*

The official method for sulfite analysis employs a colorimetric protocol based on the reaction with *p*-rosaniline, while an alternative method utilizes hot reflux distillation. The *p*-rosaniline colorimetric method is widely adopted for determining sulfites in various food products, as it relies on the formation of a colored complex between sulfur dioxide ($SO_2$) and *p*-rosaniline, the intensity of which is proportional to the sulfite concentration in the sample. According to Barros (2018), this method has been used in the analysis of sulfites in white sugar, demonstrating its applicability across different food matrices.

Conversely, reflux distillation, such as in the Monier-Williams method, is frequently used to quantify sulfites in food and beverages. However, this process can promote the extraction of other sulfur forms present in the sample, potentially leading to an overestimation of sulfite values. This occurs because, under heating and in an acidic environment, compounds like thiosulfate and sulfate can decompose, releasing additional $SO_2$ (Machado *et al.*, 2006).

$$Thyosulfate\colon\ S_2O_3^{2-} + 2\,H^+ \rightarrow SO_2(g) + S + H_2O$$

$$Polysulfides\colon\ S_x^{2-} + H^+ \rightarrow SO_2(g) + S$$

Figure 10 - Reactions involved in the decomposition of sulfur derivatives during sulfite analysis by reflux distillation

Furthermore, polysulfides and unstable sulfur species can undergo transformation, contributing to an increase in the apparent sulfite concentration. Organic compounds containing sulfur can also be broken down under these conditions, releasing more $SO_2$ than the colorimetric method would indicate (Figure 10). The Monier-Williams method may exhibit interferences due to the presence of other sulfur compounds, compromising its specificity (Nagato *et al.*, 2013; Bragagnolo *et al.*, 2001).

Thus, while the *p*-rosaniline technique offers greater selectivity for sulfite, reflux distillation can lead to the quantification of a broader spectrum of sulfur compounds. To minimize interferences and ensure higher accuracy, it is advisable to compare the results obtained by both methods, in addition to using complementary techniques such as mass spectrometry or ion chromatography. The selection of the most suitable analytical method should consider the specificity and characteristics of the sample matrix, as well as the specific objectives of the analysis.

## CONCLUSIONS

While the *p*-rosaniline method offers rapid and sensitive sulfite determination, it is susceptible to interferences, limiting its specificity. Reflux distillation, exemplified by the Monier-Williams method, provides enhanced selectivity by isolating $SO_2$ prior to titration, albeit at the cost of increased time and equipment complexity. This method revealed significantly higher sulfite concentrations in sugar samples: 17.30 mg/kg for VHP, 24.02 mg/kg for white type 1, and 26.75 mg/kg for white type 2, with white type 2 exhibiting the highest $SO_2$ content, followed by type 1, and VHP the lowest. This disparity suggests that while white sugar undergoes more intensive sulfitation, type 1 sugar benefits from more efficient sulfur removal during decantation. To reconcile these differences and ensure accurate quantification, a comparative approach is recommended. For routine quality control, the *p*-rosaniline method is suitable, but reflux distillation provides critical insights into sulfite behavior and total sulfur forms. Integrating both methodologies, supplemented by techniques like mass spectrometry or ion chromatography where necessary, enhances quality control, supports regulatory compliance, and offers a comprehensive understanding of sulfite dynamics in sugar production.

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