

### Influence of sugar concentration and sugar type on the polyphenol content and antioxidant activity

#### in spiced syrup preparation

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PAPER

### Abstract

Besides their culinary roles, spices in the eastern traditional medical practices serve as a medicinal diet therapy. The polyphenol-rich content of these spices contributes to their antioxidant properties, which convey the therapeutic elements. Traditionally, these phytonutrients are orally delivered via herbal decoction, including sugar-syrup-based decoctions. However, the impact of the addition of sugar into polyphenol content and their antioxidant activities are still insufficiently researched. Therefore, this study aimed to evaluate the influence of sugar concentration and its types (refined and unrefined) on the polyphenol content and their antioxidant activities. The results of the principal component analysis (PCA) did not exhibit any specific trend on either sugar concentration or its type. As indicative evidence, reducing sugar by less than 25% in such products can be considered for lower-calorie beverage development as a means for healthier diet choice.

Keywords: decoction syrup; low-sugar; polyphenol-sugar interaction; sugar-sweetened

### Introduction

Spices are traditionally served as culinary condiments and habitually used in Ayurveda, Persian *Tebb-e Sonnati*, Traditional Chinese Medicine (TCM), and Unani (Graeco-Arabic) medicinal preparations. Phytochemicals in spices can provide a great deal of medicinal therapy in preventing or treating common ailments. For instance, a mixture of cinnamon, clove, ginger, allspice, nutmeg, and star anise is used in the sugar-sweetened beverage for diet therapy in alleviating metabolic syndrome (Block, 2015). Their phytotherapeutic benefits are administered mostly in crude forms, either orally as infusions (herbal teas), tinctures (alcoholic extracts), decoctions (boiled extracts), and syrups (extracts of herbs made with syrup or honey), or topically applied as poultices, balms, and essential oils (Ahmad *et al.*, 2016; Ramalingum and Mahomoodally, 2014). Among the common liquid forms, *Sharbat* or *Sérbét*, which has the Arabic root word *shariba* or "to drink," is made from either decoction, infusion of herbal remedies, fruit juice, or flower petal mixtures. There are various types of *Sharbat* preparation, depending on the type of the substrate to be extracted. Commonly, dry substrates, like most culinary spices, are boiled until one-third of the water is left and allowed to cool before being filtered. The filtered decoction is usually sugar sweetened with two to three parts and further boiled to obtain a syrupy consistency. Addition of sugar to the phytochemical extraction step is relatively similar to the fruit osmotic dehydration technique, whereby sugar syrup is used as an osmotic agent (Pereira et al., 2014) to produce an aromatic and flavorful herbal syrup or extract (Geng and Zhou, 2010). The range of osmotic agent additions were from 2 to 50% of the herbal substrate, but the study did not indicate as to when the sugar should be added. This syrup extraction was traditionally meant to improve the flavor and aroma of the herbal extract but was not intended to enhance the extractability of antioxidant compounds or specifically the polyphenols. Previously, it was known that a hot aqueous extract of several spices (non-sugar addition) had a high antioxidant activity that was probably due to the phenolic and flavonoid compounds (Kim et al., 2011). Later, a study revealed that the addition of sugar before or during processing might play a significant role in enhancing the polyphenol extraction, and simultaneously the antioxidant activities instead of improving only the gastronomic qualities (Loncaric et al., 2014). However, studies that evaluate the impact of sugar addition on polyphenols' content and their antioxidant activities are still limited to ensure this positive enhancement. The latest findings showed that there were only slight polyphenol losses during food processing (Szymanowska et al., 2017; Zeng et al., 2017) to unapprehensive changes in antioxidant capacities (Baroni et al., 2018) upon the introduction of sugar, which was probably due to some degree of protective mechanism. A similar mechanism was observed in chokeberry juice that was added with polysaccharide as the clarifying agent (Lachowicz et al., 2018). However, the polyphenolic content became less as soon as the natural sedimentation appeared, resulting in polyphenol binding to polysaccharides.

A list of sugar-loaded syrups showed promising therapeutic potentials as novel sources of antioxidant polyphenols. Among the examples are the traditional prickly pear (Opuntia ficus indica) and the pink Tecoma (Tabebuia impetiginosa) inner-bark syrups were advocated for their anti-tumor potential (Dhaouadi et al., 2013; Pires et al., 2015), and the English plantain (Plantago lanceolate) leaf syrup was recommended for combating common cold (Mansoor et al., 2017). A polyphenol-rich syrup can potentially be a novel functional ingredient like the bog bilberry (Vaccinium uliginosum) syrup (Malvidin-3-O-glucoside, petunidin-3-O-glucoside, and delphinium-3-O-glucoside), which is the chief flavonoid glucoside that is used to enrich wine for antioxidant capacities (Liu et al., 2015). Carbohydrates can enhance the bioactivity and the bioavailability of polyphenol compounds through increased fermentation by microbiota in the large intestine, especially the oligosaccharides (Zhang et al., 2014). However, the primary concern is monosaccharide and disaccharide, which are readily absorbed and digested in the small intestine and may cause an alarming glycemic response in patients with metabolic syndrome.

Nevertheless, the negative health impact of commonly consumed sugary beverages on the risk factors for metabolic syndrome cannot be validated with adequate clinical evidence (Angelopoulos *et al.*, 2016; Della Corte *et al.*, 2018) to confirm that the added sugar products are the chief culprits for the metabolic imbalance. While it is health-wise beneficial to consume added sugars beyond the average level, the reduction in sugar intake accompanied by restricted calorie intake may be more beneficial (Rippe and Angelopoulos, 2016). Therefore, this preliminary study was to evaluate the effect of sugar concentration and the sugar type by examining their influence on polyphenol contents and their antioxidant activities.

### **Material and Method**

Four culinary spices comprising Chinese cinnamon (*Cinnamomum cassia*), dried ginger (*Zingiber officinale*), green cardamom (*Elettaria cardamomum*), sweet fennel (*Foeniculum vulgare*), and six types of sugar, namely, refined white sugar (RWS), filtered sugarcane brown sugars (FBS), unfiltered sugarcane brown sugar (NBS), organic molasses (OMO), Javanese coconut sugar (JCS), and Malaccan coconut sugar (MCS) were acquired from Jaya Grocer store, Bangi Gateway.

#### Spiced syrup preparation

Dirt and debris were removed from the spices by cleaning under running tap water, and the excess water was drained before they were air-dried. A mixture of spices, comprising 10% (w/v) cinnamon and 5% (w/v) other spices, was added to boiling sugar syrup (110°C) before it was simmered for 240 min at 80°C. The spice mixture was directly used without pounding into smaller sizes based on a traditional technique to avoid a strong flavor. The spiced syrup was prepared (Figure 1) according to a traditional recipe, whereby sugars were added to filtered water at ratios of 1:2 kg/L, 1:3 kg/L, 1:4 kg/L, and 1:6 kg/L to produce syrups at the corresponding concentrations (Table 1). The range of sugar additions was according to traditional practices in preparing a sugar-based decoction. However, the most common practice is to mix one part of sugar with four parts of water (25%, w/v). The non-spiced 25% sugar syrups (all selected sugar types) were boiled and simmered in the same way as the spiced syrup was prepared and were considered as the positive control (control, CTRL [sugar type]), and the spice decoction without sugar syrup (0% sugar) was considered as the negative control. The spiced syrup was then filtered with a clean muslin cloth before being bottled and



Figure 1. Flow chart of spiced syrup processing

Table 1.	Sugar ratio	to sugar	percentage	conversion.
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Sugar to water ratio (kg/L)	Sugar used (g/1000 mL)	Sugar percentage, % (w/v)
1:2	500.0	50.0
1:3	333.3	33.3
1:4	250.0	25.0
1:6	166.7	16.7

stored at room temperature until use. Each spiced syrup was prepared in triplicates. The uncertainty due to spice sampling heterogeneity was kept below 13%, as recommended by Bodnar *et al.* (2013).

#### Determination of the total phenolic content

The Folin–Ciocalteu (FC) method (Singleton et al., 1999) was used to quantify the phenolic content with slight modification on the reactant volumes for 96-well microplate suitability. A hundred microliters of 10% FC reagent were mixed with 20 µL of blank (deionized water)/ standard/filtered syrup (diluted 50-fold with deionized water). After 5 min, 80 µL of 7.5% sodium carbonate was added and incubated at 37°C for 120 min. This spectrophotometric measurement was carried out at  $\lambda$ = 765 nm (Spectrostar Nano, BMG Labtech, Germany). The quantification of TPC by using the calibration curve of gallic acid was calculated by MARS Data Analysis Software Version 2.01. The concentration for the calibration curve ranged from 250 to 4000 ppm. The results were expressed as micrograms of gallic acid equivalents (GAE)/mL of syrup.

#### Antioxidant activity determination

Multiple spectrometric techniques were employed to evaluate the spice polyphenol antioxidant activities by three different mechanisms: organic radical scavenging, ferric reducing capacity, and peroxyl radical scavenging.

It was essential to evaluate the antioxidative compounds by using various techniques due to the unique identity of each polyphenol with different action mechanisms against oxidative substances. With these multiple antioxidant determinations, an almost complete profile of antioxidants can be attained.

# The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were determined according to Kodama *et al.* (2010). The 0.5 mM DPPH was pre-adjusted with methanol (1.00  $\pm$  0.1 absorbance unit) before being mixed with diluted syrup (20-fold with distilled water) at a ratio of 9:1. After 30 min, the absorbance of mixtures was measured at 515 nm, and the DPPH radical scavenging activities were calculated as mM Trolox equivalent (TE)/mL of syrup.

### Ferric reducing antioxidant power assay

A ferric reducing antioxidant power (FRAP) assay of spiced syrup was based on Musa *et al.* (2011), which required the FRAP reagent to be freshly prepared by sequentially mixing one part of 20 mM FeCl<sub>3</sub>.  $6H_2O$  with one part of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and thoroughly mixing with 10 parts of 300 mM acetate buffer, at pH 3.6. A freshly prepared FRAP reagent at a 1950 µL was mixed with 50 µL diluted syrup before a 30-min incubation at room temperature. The absorbance changes against the blank (distilled water) were measured at 595 nm. The Trolox calibration curve (3.125 mM to 100 mM) was used to equivalently estimate the FRAP reducing capacities, with results expressed as mM Trolox equivalents (TE)/mL of syrup.

#### Oxygen radical absorbance capacity assay

For the oxygen radical absorbance capacity (ORAC) value determinations, 150  $\mu$ L of 10 nM fluorescein solution was mixed with 25  $\mu$ L diluted syrup in a 96-well black microplate, as described by Payne *et al.* (2013). After incubation for 30 min at 37°C, the fluorescence degradation measurements were executed at the emission and excitation of 520 nm and 485 nm, respectively, by using a microplate reader (FLUORstar Omega, BMG Labtech, Germany). Automatic injection of 240 mM AAPH (25  $\mu$ L) at the fourth cycle was made before measurement resumption, and the calculated results were expressed as  $\mu$ M Trolox equivalents (TE)/mL of syrup.

#### Statistical analysis

Data collected as means of triplicate readings were subjected to analysis of covariance (ANCOVA), correlation analysis by Pearson's correlation coefficient, and data analysis by principal component analysis (PCA) by using XLSTAT Premium 2018.1.4913 (Addinsoft Inc., New York, NY, USA). The ANCOVA was applied to evaluate whether TPCs were varied with sugar concentrations (quantitative) and types (qualitative), and verify a sensible linear model. The exploratory statistical tool, PCA, was applied to observe any typical or divergent features or degree of similarities among a set of variables.

#### **Results and Discussions**

# Effect of sugar concentration and sugar type on the TPC of spiced syrups

Figure 2 shows that all CTRL sugars had a TPC that ranged from 500 to 1000 GAE mg/mL, except for RWS, which was the least with < 500 GAE mg/mL. Java coconut sugar, JCS had the highest TPC among the selected sugar types of approximately 1000 GAE mg/mL. Most brown or unrefined sugars contained a significant amount of polyphenol (P < 0.05), compared to refined sugar (Kongkaew *et al.*, 2014; Choong *et al.*, 2016). Brown sugar from sugarcane and coconut are commonly used in traditional medicine preparation as a sweetener because it is believed to possess therapeutic benefits. However, the 0% sugar decoction had 2-fold to 5-fold higher TPC than each CTRL sugar, and showed that sugar syrup of any type could contribute to a smaller portion of TPC value in the spiced syrup. Overall, the higher the sugar concentration, the higher was the polyphenol content, except for OMO, which showed an opposite trend. This contradictory effect on OMO might be attributed to the OMO polyphenol antagonistic effect, reflecting the reduction of content. Meanwhile, in the RWS syrup, the TPC showed a unique bell-shaped trend, whereby intermediate sugar concentrations of 25 and 33.3% were exhibited among the highest contents (> 2500 GAE mg/mL). At higher sugar concentrations, the TPC high and low values were possibly due to the osmotic pressure, which allowed for desorption or prevention of spice polyphenols into the sugar syrup. It was postulated that the TPC decrement in the spiced syrup as the sugar concentration decreased was due to spice polyphenol degradation in the low solute matrix. However, the enhancement of the polyphenolic content in pumpkin by using concentrated flowering quince juice was attributed to the penetration of the polyphenol-rich osmotic solution into the pumpkin, which provided some stability (Lech et al., 2018). The inconsistent variance among TPC in a syrup of different sugar concentrations and types might occur due to the interaction of Folin-Ciocalteu reagents with any reducing or readily oxidizable substances of nonphenolic compounds, causing an aberration of the total phenolic content (Essawet et al., 2015; Ho et al., 2017; Ramachandran and Nagarajan, 2014). It was postulated that different profiles of monosaccharide and disaccharide could play some interaction with the spice-polyphenols via a covalent or non-covalent bond, and thus, reflecting some variation in free polyphenol content.

The ANCOVA analysis showed that 63% of TPC variability (Table 2) was explained by sugar concentrations and types. However, the latter variability and interaction between sugar concentrations and types did not



Figure 2. Total phenolic content (TPC) of spiced syrups prepared from different type of sugars and concentrations.

Table 2. Summary of ANCOVA for all variables.

		TPC	DPPH SC	FRAP RC	ORAC V
R²		0.627	0.403	0.273	0.439
Pr > F		< 0.0001	< 0.0001	0.067	0.000
Sugar concentration (%)	F		0.730	10.740	
	Pr > F		0.401	0.003	
Sugar type	F	1.814	2.726		1.106
	Pr > F	0.150	0.018		0.395
Sugar concentration (%)	F	2.234		1.226	1.262
* Sugar type	Pr > F	0.084		0.321	0.320

ANCOVA, analysis of covariance; TPC, total phenolic content; ORAC, oxygen radical absorbance capacity; DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power.

significantly explain the TPC variability. Furthermore, the equation of the TPC linear model is proposed,

$$TPC = 733.60 \times Type \text{ of Sugar-C-FBS} + 1070.85$$
  
 
$$\times Type \text{ of Sugar-C-JCS} + 602.73$$
  
 
$$\times Type \text{ of Sugar-C-MCS} + 872.03$$
  
 
$$\times Type \text{ of Sugar-C-NBS} + 880.13$$
(1)

Since the type of sugar did not carry significant information, the proposed model (Equation 1) was rejected. Based on Type 3 sum of squares, the interaction variable (type of sugar  $\times$  sugar concentration) was the most influential contributor to the insignificant variation. Therefore, RWS can still be utilized in the production of the spiced syrup without a considerable loss in the TPC. One can avoid brown sugar in the mass production of the spiced syrup as it was uneconomically feasible.

# Antioxidant capacities (DPPH scavenging, FRAP reducing activities, and ORAC values) of spiced syrups

From the summary of variable analysis (Table 2), 44% of the ORAC value variability was explained by the two explanatory variables (sugar concentration and type). The variability of the ORAC values in the spiced syrup was similar to TPC variability, which was not significantly explained by the type of sugar and the interaction variables of sugar concentration and sugar type. It may explain that the TPC content may have a corresponding influence on the ORAC values. All the spiced sugar syrups showed decrement trends in the ORAC values, excluding three negligible samples that showed increments (Figure 3). Meanwhile, the DPPH scavenging and the FRAP reducing capacities had 40 and 27% variabilities, respectively, explained. The sugar concentration in DPPH

scavenging capacity (P > 0.05). However, the type of sugar played an influential role in explaining the variation in the capacity based on Type 3 sum of squares. It contradicted the FRAP reducing capacity, whereby the sugar concentration significantly influenced its variability.

The DPPH scavenging capacity of the spiced syrup showed decreasing trends in the RWS (0.6 to 72%) but the opposite trend in the MCS-based spiced syrup (31–53%), as compared to the control zero-sugar spiced decoction (Figure 4). The rest of the four-brown sugar-based decoctions exhibited mixed trends in the scavenging capacities. All FRAP reducing capacities showed reductions as negatively influenced by sugar, excluding two samples from the different types of sugar (Figure 5).

# Visualization of the correlations between variables by using PCA

For distinguishing the spiced syrup-based TPC and antioxidant activities, the PCA was employed on the Pearson correlation matrix. As a classification/discrimination method, PCA permits a simplified data representation over a complex data matrix of the spiced syrup with different sugar concentrations and types of sugar use. The first two principal components (PC), PC1 and PC2, explained a total of 78.4% variance (Figure 6). Both PCs were almost equally explained, whereby the initial PC, explained up to 41.7% and the latter up to 36.7%. PC1 has a strong positive correlation with the TPC and all antioxidant variables, whereby the ORAC values have the highest correlation with the TPC (P < 0.514) (Table 3). The DPPH scavenging and FRAP reducing capacities have the least correlation with the other two variables and are negatively correlated with each other (P < -0.465).



Figure 3. ORAC values of spiced syrups prepared from different type of sugar and concentration.



Figure 4. DPPH radical scavenging activities of spiced syrups prepared from different type of sugar and concentration.



Figure 5. FRAP values of spiced syrups prepared from different type of sugar and concentrations.

From the biplot (Figure 6), there was no specific classification based on either concentration of sugar or the type of sugar exhibited on both the PCs. However, two clusters can be observed in the positive and negative loadings of PC2 without specific classification in each cluster. The PCA analysis results showed that either sugar concentration or sugar type significantly influences the TPC and their antioxidant activities.

Only hard ingredients, either from bark, seed, or heartwood, are traditionally subjected to simmering. In contrast, the soft or leafy ingredients are infused in boiled water to extract bioactive compounds. The duration of the

Table 3. Correlation matrix (Pearson [n – 1]).

Variables	TPC	DPPH SC	FRAP RC	ORAC V
TPC	1	0.201	0.255	0.514
DPPH SC	0.201	1	-0.465	0.220
FRAP RC	0.255	-0.465	1	0.156
ORAC V	0.514	0.220	0.156	1

Values in bold are different from 0 with a significance level alpha = 0. ANCOVA, analysis of covariance; TPC, total phenolic content; ORAC, oxygen radical absorbance capacity; DPPH, 2, 2-Diphenyl-1picrylhydrazyl; FRAP, ferric reducing antioxidant power. former may take hours as compared to the latter, which only takes a few minutes. The decoction process started after the sugar syrup was boiled and the spices were added and terminated after 4 h of simmering. The spices were introduced into the boiled sugar syrup to obtain the glassy clear syrup in culinary practice. The point at which the sugar was added did not significantly impact taste (Madhavamenon and Maliakel, 2015). However, from the study observation, the addition of sugar toward the end of the decoction process only retained the liquid turbidity, which was visually less appealing.

Previous studies showed that mild thermal exposure, such as in the fortification of sugar syrup with functional fruitbased ingredients, retained its high polyphenol content (Tarko *et al.*, 2015). Those that were traditionally prepared fruit-based beverages also showed more pronounced antioxidant capacities than the commercially processed juice (Marjanović *et al.*, 2015). A traditional Calabrian fig syrup made from fresh figs' (*Ficus carica*) aqueous slurry, prolong boiling until the syrup concentrate has a high polyphenol content (3.92 mg of GAE per gram of syrup) with desirable sensorial qualities (Puoci *et al.*, 2011). It seemed to be not much affected by the lengthy thermal treatment. Therefore, the effect of the thermal treatment on the polyphenol content of the spiced syrup was not carried out. The decrement trend in radical scavenging capacities



Figure 6. PCA biplots of spiced syrups made from different type of sugar and concentrations versus TPC and antioxidant parameters.

might be attributed to the glycosidic bond formation through the condensation reaction between a hydroxyl group of sucrose molecule and the hydroxyl group of phenolic compounds to form glycoside compounds. However, the increments were probably the result of phenolic reduced-form compounds due to the oxidation of phenolic compounds and sucrose molecules (Shalaby *et al.*, 2016). In addition, Jlassi *et al.* (2016) suggested that the dose-dependent manners of the polyphenol bioactivities may vary due to the polyphenol functional site reaction with ionized sucrose. Besides, the polyphenol profile may influence disaccharide action and give variability in antioxidant activities (Pengseng *et al.*, 2011).

Sugar-sweetened beverage consumption still gives a stir to the national health issues. Simultaneously, polyphenol-rich food intakes are linked with a positive impact on consumers' health. However, sugar addition to polyphenol-rich food products, especially beverages, can change the health benefits. Some suggested that the presence of sugars generally increases the bioavailability of polyphenols, but product formulation may influence the sugar impact to a certain degree (Ackar et al., 2013). This study observed a specific range of sugar concentration and sugar type that accounts for a certain extent of the TPC. These two variables may introduce different profiles of disaccharides, which in turn react to a certain extent. It was not only the type of disaccharide that was reported to have an essential impact on phenolics but also their chemical isomers since the chemical behavior can be different in complex food matrices (Zlatić et al., 2017). For example, maltose and trehalose are isomers of sucrose. The latter isomer has a more hydrophilic site than the former, leading to a higher interaction with hydrophilic polyphenols. In the study spice syrup, hydroxycinnamic acids derived from cinnamon, which was hydrophilic (Teixeira et al., 2013), probably had more sugar interaction. In addition to the saccharide structure-related factors, evidence showed that the polyphenol interaction with saccharides was influenced by the polyphenol molecular weight (monomeric or polymeric) (Amoako and Awika, 2016). It was found that polymeric polyphenol (molecular weight, MW > 1000) polysaccharides via hydrogen bonding and hydrophobic interactions formed nondigestible complexes. This positive interaction can reduce calorie density and may help modulate glucose metabolism. Therefore, future research on the consequences of polyphenol-sugar interaction should uncover a positive impact on health.

In the food matrix, polyphenols can have either a covalent or noncovalent association with polysaccharides. A combination of hydrophobic interaction and hydrogen bond (between the phenolic hydroxyl group and the polysaccharide oxygen atom) can result in a noncovalent association. Simultaneously, the covalent association involved the oxidation of phenolic compounds (Le Bourvellec and Renard, 2012), which probably occurred in the spiced syrups and highly corresponded to the low antioxidant activities. It showed that both types of association have a determinant effect on the quality level of polyphenol-rich beverages. Sugars engage in the stability of polyphenols in the form of conjugated sugar or glycoside via a glycosidic bond (excluding the subclass of catechins) to one or more hydroxyl groups (Martin, 2009). Therefore, the addition of sugar may inhibit the autoxidation of polyphenols and contribute to high antioxidant activities in some spiced syrups. However, the profound antioxidant activities were not solely contributed by inhibition of autoxidation. It may partially be due to the advantage of additive or synergistic effect of different phenolics, flavonoids, reducing sugars, and vitamin C profiles like in the must of dried grapes (Peinado et al., 2010), whereby an antagonist effect may be found in the spiced syrup for the reduced antioxidant activities. The glucoside forms (sugar conjugated) are likely to offer more therapeutic benefits than the aglycones (nonsugar conjugated polyphenol), as they are more bioaccessible, bioavailable, and stable under in vivo conditions (Fernandes et al., 2017). In addition, the sugar moiety of glycoside can enhance the bioavailability, but dimerization weakens the ability. However, glycosides are usually weaker in vitro antioxidants than aglycones (Shashank and Pandey, 2013); this explains why some spiced syrups have lower antioxidant activities than the zero-sugar spiced decoction.

# Conclusions

Various sugar concentrations and different types of sugar used in the extraction of polyphenols to produce the spiced syrup may influence the TPC and antioxidant activities to a particular extent with mixed variabilities, as observed from the results of the ANCOVA and the PCA. The weaving trends may uniquely contribute to the different profile components of sugar and water-soluble phytochemicals from the spice mixtures. These biochemical complexities of molecular weight and structurerelated reaction produced a different level of antioxidant activities. From the compiled results of the ANCOVA and the PCA, it can be suggested that a sugar concentration of less than 25% is adequate to prepare the spiced syrup with many antioxidant activities, even while using the RWS. Moreover, further studies are required to better conclude the sugar-polyphenol interaction as in vivo.

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