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PHYSICOCHEMICAL PROPERTIES OF CAROB SYRUP REVEAL D-PINITOL AS AN INDEX OF SUCROSE SUPPLEMENTATION

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Abstract: *The carob syrup has been attracted the interest of consumers as an alternative sweetener due to its superiority against refined ones. Bioactive phytochemicals and polyols, vitamins and minerals are present in carob syrup; whereas sucrose, fructose and glucose are the main sugars. Although the consumers prefer carob syrup for its special composition, the sucrose supplementation in carob syrup is a common adulteration abating its health benefits. The detection of adulteration in natural sweeteners and honey is usually required time-consuming and advanced analytical techniques. In the present study, the physicochemical properties of carob syrup were studied to pinpoint physicochemical parameters for detection of sucrose supplementation. More specific, moisture, fats, pH, color parameters and total soluble solids were determined. Furthermore, sugar composition of carob syrups was studied using chromatographic separation. Finally, the phenolic fraction and antioxidant activity of carob syrups were estimated. Results showed significant differences in total soluble solids and moisture contents, but these parameters are linked with its preparation procedure. Furthermore, an unexpected fluctuation was observed for D-pinitol and total phenolics contents. Thus, both parameters were determined in genuine and sucrose-supplemented carob syrups. Based on our findings the sucrose supplementation can be detected by D-pinitol content and sucrose to D-pinitol ratio. Therefore, the present work demonstrated the potential of a simple chromatographic separation to detect the adulteration in a traditional Mediterranean product.*

Keywords*: adulteration, sugars, D-pinitol, carob syrup, chromatography, phenolics*

1. Introduction

The carob syrup is a traditional sweetener native to the Mediterranean basin. It comprises a high proportion of sugars namely sucrose, fructose and glucose (> 65%) and small amounts of polyols [1]. It also contains additional nutritive compounds such as polyphenols, minerals, proteins and fibres. Furthermore, the energy density and glycaemic index of carob syrup is lower than refined sweeteners as sucrose [2]. Moreover, the traditional medicine describes the use of carob syrup to treat gastrointestinal and venereal diseases and to control rheumatic disorders [3-4]; whereas the health effects of many carob syrup constituents like phenolic compounds, fibres and polyols have been documented [5-6]. The aforementioned benefits of carob syrup and the increased demand for natural and healthy sweeteners have attracted the interest of consumers.

The carob syrup is produced according to traditional method in Cyprus. In particular, the chopped carob fruits were suspended with slightly warm water (1/3, w/v) to recover sugars as carob juice. Subsequently, the carob juice is concentrated via boiling at least 66.5 °Brix. This procedure is guided to the low yield of carob syrup due to the use of slightly warm water at the stage of maceration. The use of hot water increases the yield of sugar contents but it also recovers

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undesired astringent tannins. Thus, some patents have been registered in order to transcend this drawback but the utilization of expensive chromatographic techniques is required. Therefore, the adulteration of carob syrup using sucrose is widespread increasing the yield of syrup. Carob fruits and its derivatives contain significant amounts of sucrose [1, 5], thus, it is difficult to detect the sucrose supplementation in carob syrup. The objective of the present study was to monitor physicochemical properties of Cypriot carob syrup to discovery useful indexes for adulteration. Then, the most promising properties were used to compare genuine carob syrups and sucrose-supplemented ones.

2. Matherials and methods

Carob syrups

At first, twenty-five commercial carob syrups were purchased from local markets for two successive years. For the preparation of genuine and sucrose-supplemented carob syrups, Tilliria' carob fruits were harvested at maturity stage from Limassol and Pafos Districts. Fruits were transferred to the laboratory and used for the preparation of carob syrup according to traditional method used by Cypriot families (**Figure 1**).

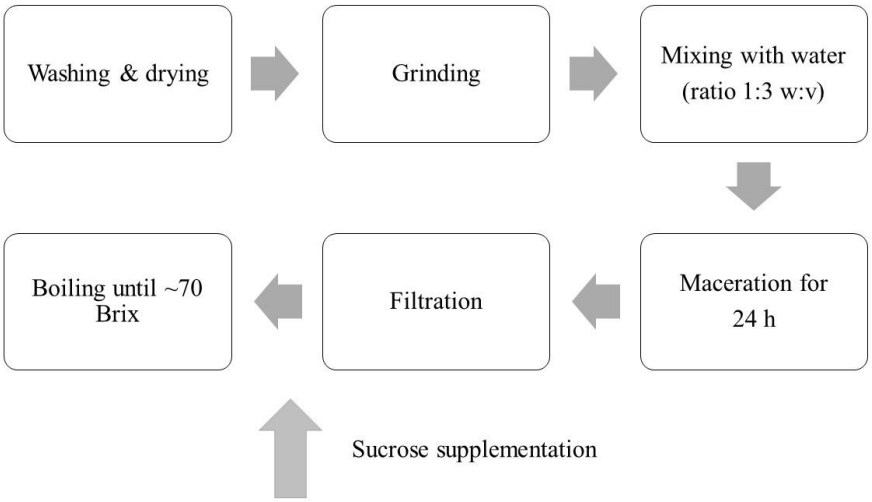

Fig. 1. **Preparation process of carob syrups according to the traditional method.**

Proximate analysis

The moisture contents of carob syrups were measured by a moisture analyzer (Kern MLS-50-3, KERN & Sohn GmbH, Germany). The pH of samples was also measured by a benchtop pH meter (HI2211, Hanna Instruments, USA).

Fat contents were determined using Bligh & Dyer method. Total soluble solids (Brix) were determined using a portable digital refractometer (DR103L, Sun Instruments Corp. USA).

Color

Carob syrups were placed in a uniform layer (0.5 cm) on a 5 cm diameter Petri dish with the employment of a reflection colorimeter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The measurement head was over the dishes. Color was calculated using the CIE-L* a* b* uniform colour space (CIE-Lab), where L* indicates lightness, a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis.

Total phenolic content and antioxidant activity

Total phenolics content of carob syrups was estimated by a colorimetric assay based on previous workd with some slight modifications [7]. More specific, 5 g of syrup were shaken with 5 mL of ethyl acetate for 5 min. Then, the mixture was centrifuged for 10 min at 4700 rpm. The procedure was repeated three times and the filtrates were merged and used for total phenolic content and antioxidant activity assays. A 400 μL aliquot of sample was mixed with 500 μL of Folin-Ciocalteu reagent and 4.6 mL of deionised water. After mixing the contents for 3 min, 1 mL of saturated sodium carbonate and 3.5 mL of deionised water were added. Samples were left to stand at room temperature for 60 min. Absorbance measurements were taken at 725 nm. A standard curve of gallic acid was prepared and results expressed as mg gallic acid equivalents (GAE) 100 g^{-1} carob syrup. Ethyl acetate extracts of carob syrup was also used for the determination of antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. In particular, 30 μL of extracts was reacted with 2 mL of DPPH solution (0.3 mM) for 30 min at dark. Subsequently, the absorbance of mixture was read at 517 nm and results are expresses as a *μmol* ascorbic acid equivalents (AAE) 100 g^{-1} carob syrup by the preparation a standard curve of pure ascorbic acid [8].

Sugar composition

The samples were prepared according to previous work [1]. The sugar composition of carob syrups was analysed on a Waters series HPLC (Waters Corporation, Milford, Ireland) system equipped with vacuum degasser, binary pump, autosampler, thermostated column compartment, refractive index detector and Empower software for data collection and analysis. The diluted samples were loaded on an Aminex HPX-87P column (300 mm × 7.8 mm × 9 μm) at 80 °C. The flow rate was set at 0.5 mL min^{-1} and the injection volume was 20 μL as previous study describes for the separation of sucrose, glucose, fructose and D-pinitol in alfalfa biomass [9]. Quantitation was performed by standard curves generated by chromatographic analysis of the standard solutions of the respective substances at various concentrations

3. Results and discussion

Physicochemical properties of carob syrups

Physicochemical properties of carob syrups showed considerable differences between commercial samples. **Table 1** summarizes results of chemical analysis of twenty-five Cypriot carob syrup that are available in local market.

Table 1.

Physico-chemical properties of commercial carob syrups produced in Cyprus

Parameter	Mean	Median	Range
Moisture (%)	23.6	24.2	19.1-29.4
Fat (%)	0.17	0.17	0.11-0.22
pH	4.57	4.58	4.39-4.80
L*	21.32	21.34	21.03-21.64
a*	0.34	0.18	0.11-1.20
b*	0.57	0.58	0.43-0.71
°Brix	74.2	73.7	69.3-80.9
Total phenolics*	912	960	300-1250
DPPH activity**	129	129	103-161

*Total phenolics are expressed as mg gallic acid equivalents 100 g^{-1} carob syrup

** DPPH activity is measured as μmol ascorbic acid equivalents 100 g^{-1} carob syrup

Total soluble solids of carob syrup were ranged between 69.3 Brix and 80.9 Brix; the median value of total soluble solids was found at 73.7 Brix. Previous studies also described similar total soluble solids for Tunisian and Turkish carob molasses [1, 10]. Besides, the above concentration of sugars is required to avoid the growth of foodborne microorganisms. The moisture contents of carob syrups were inversely related with sugar contents; the median value of moisture was 24.2% w/w. The moisture content is linked with the evaporation process to concentrate carob juice. Results also showed a low-fat content of carob syrups; the fat content was between 0.11 % w/w and 0.22 % w/w. It is expected as carob fruit contain small amounts of fats (~0.60% w/w) and the water is used as an extractor medium for recovery of hydrophilic sugars [11]. The pH of carob syrup is linked with soluble organic acids; there are no significant differences between carob syrups. Similar pH values have also been found in Tunisian carob molasses [10].

Afterwards, the color values L∗, a∗, and b∗ were measured to designate the color space of carob syrups. More specific, the color is expressed as three values: L* for the lightness from black (0) to white (100), a* from green (−) to red (+), and b* from blue (−) to yellow (+). Results demonstrated that only value a* had significant differences between studied carob syrups. In particular, carob syrups with high sugar contents had higher values of a*. On contrary, there are no considerable differences between values for L* and b* for carob syrup studied.

Carob syrups also contains significant amounts of phenolic compounds; whereas the gallic acid is the major constituent of phenolic fraction (>80%) [12]. Results also showed a high phenolic content in carob syrup; its mean value was 960 mg gallic acid equivalents 100 g^{-1}. However, a small number of samples had 3-fold lower phenolic contents. The latter cannot be explained by the diversity of phenolics in carob fruit. Furthermore, the antioxidant activity of carob syrups was determined with the employment of DPPH assay. The antioxidant activity of carob syrups was ranged from 103 to 161 μmol ascorbic acid equivalents 100 g^{-1}. This assay revealed shorter variation between carob syrups than Folin-Ciocalteu assay. It can be attributed to the chemistry behind of each assay. The DPPH assay measures the ability of antioxidants to scavenge free radicals; whereas Folin-Ciocalteu assay is based on the transfer of electrons in alkaline solution from the phenolic compounds to phosphomolybdic/ phosphotungstic acid complexes [8, 13].

The chromatographic separation of sugars and D-pinitol revealed interesting variances in carob syrups. Sucrose was the main sugar in all samples, followed by fructose, glucose and D-pinitol. The sugar composition is in line with previous studies for Mediterranean carob syrups [1, 10]. Results demonstrated a fluctuation range of 18% for sucrose, 32% for fructose, 34% for glucose, and 75% for D-pinitol (**Table 2**). The differences in D-pinitol in carob syrup are unexpected as a recent study reported that the D-pinitol content in Cypriot carob varieties was from 53.20 mg g^{-1} and 54.58 mg g^{-1} [14].

Taking into consideration that there are minor changes in the production of carob syrup, the D-pinitol content alone and/or in combination with sucrose content can be used as an index of sucrose supplementation in carob syrups. Thus, we prepared genuine and sucrose-supplemented carob syrups to investigate the above hypothesis.

Table 2.

Sugar composition of commercial carob syrups produced in Cyprus.

Compound	Mean	Median	Range
Sucrose	368	374	332-404
Fructose	158	156	125-184
Glucose	151	148	115-189
D-pinitol	60	65	18-70

Results are expressed as g 100 g^{-1} carob syrup

Comparison D-pinitol and sucrose content in carob syrups

In a next step, genuine and sucrose-supplemented carob syrups were prepared and their sugar composition was studied. Sucrose as an easy-available and low-cost sweetener, was added in carob juice to increase the yield of syrup. This addition of sucrose in natural sweetener as honey maple syrup as well as fruit juices is a common practice [15-17]. The supplemented sucrose was 27% and 42% of final sugar content of carob syrups. *Table 3* demonstrates a significant impact of sucrose on the chemical composition of carob syrup.

Table 3.

Effect of sucrose supplementation on sugar composition and total phenolic content in traditional made carob syrups

	Pafos	Pafos 27%	Pafos 42%	Lemesos	Lemesos 27%	Lemesos 42%
Sucrose*	378.6±21.3	451.2±10.3	512±19.3	362.9±9.3	461.3±30.5	505.3±19.8
Fructose*	138.5±6.2	109.3±3.8	84.3±6.3	162.4±5.8	116.1±14.9	102.1±7.2
Glucose*	151.2±7.4	116.8±9.3	93.3±4.8	141.3±106.6	109.6±2.9	89.6±4.7
D-pinitol*	66.7±4.2	50.6±3.3	40.4±2.1	63.9±3.0	48.5±1.8	38.9±2.3
Total Sugars*	**735.0**	**727.9**	**730.2**	**730.5**	**735.3**	**735.9**
Total phenolics**	1021±72	863±49	680±33	947±52	725±48	592±41

*Results are expressed as g 100 g^{-1} carob syrup

** Total phenolics are expressed as mg gallic acid equivalents 100 g^{-1} carob syrup

In sucrose-supplements syrups, the D-pinitol content decreased significantly; while the concentration of sucrose had a noteworthy increase. D-pinitol content is significant lower in sucrose supplemented syrups than genuine ones. The decrease in D-pinitol was 22-25% and 38-40% compared to genuine syrups when the added sugar was 27% and 42%, respectively. Furthermore, the ratio sucrose to D-pinitol content also can be used to detect the sucrose supplementation. The above ratio is approximately 5.7 in genuine syrups; whereas the ratio sucrose to D-pinitol grows up to 13 for sucrose supplemented syrups. As it is expected, the addition of sucrose also causing a drop in phenolic content in carob syrups. Dhaouadi and co-workers also noticed a noteworthy decrease of phenolic content after sucrose supplementation [18]. Specially, the total phenolic contents were decreased up to 23% and 38% for 27% and 42% sucrose supplementation. Thus, this simple and rapid spectrophotometric assay can be also exploited as an additional index for sucrose supplementation.

4. Conclusion

Carob syrup is a traditional sweetener in Mediterranean basin with low glycemic index and health effects as it is consumed within their natural matrix. However, the carob syrup fraud and authenticity are an emerging issue as the addition of cheap

sweeteners eg sucrose has been often used. In the present study, a knowledge base of physiochemical properties of Cypriot carob syrup was build. Our findings highlight that D-pinitol content, sucrose to D-pinitol ratio and total phenolic content can be utilized to detect sucrose supplementation in carob syrups. Nevertheless, an integrated study with samples from whole island for three successive years is required to legislate national regulations and standards for this traditional product.

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