

POLARIMETRY AND SPECTROPHOTOMETRY TO DETECT ADULTERATION IN COMMERCIAL CAROB MOLASSES IN LEBANON

Ossama Dimassi^{1*}▪, Mohammad Rached²▪, Rola Fawaz¹ and Raymound Akiki¹

¹Lebanese International University, Department of Nutrition and Food Sciences, Mouseitbeh,
PO Box: 146404, Mazraa, Beirut – Lebanon

²Lebanese International University, Department of Biomedical Sciences, Mouseitbeh,
PO Box: 146404, Mazraa, Beirut – Lebanon

▪Authors contribute equally

E-mail: odimassi@gmail.com (*Corresponding author)

Abstract: Carob molasses is a traditional sweet consumed in Middle Eastern countries. Processing of carob molasses differs in each and every country and the presses of carob molasses vary within the same country. Carob molasses main processing steps start from storing and grinding and de-seeding of whole pods followed by soaking with water and ending with concentrating the pure extracted carob permeate using heat. Since carob molasses as a product is sold under the claim that it has no added sugar and free of any foreign material there is a need to develop easy to conduct and low cost methods as a tool to detect these adulterations. The parameters chosen to check for adulteration of commercial carob molasses with either sucrose or glucose syrup were angle of rotation (AR) (by polarimeter) and absorbance (Ab) at $\lambda=302$ (by spectrophotometer). Base line was calculated using values of AR and Ab of 0% till 50% degree of adulteration (DA) with 5% interval and 100% adulteration using sucrose and glucose syrup. Least significant difference was calculated and accordingly the means of AR and Ab. As for Ab value it was found that at $\lambda=302$ nm, the difference between the pure carob molasses and adulterated carob molasses using glucose syrup and sucrose started being significant at level of 5% and 30% respectively. When the AR and Ab values of 18 commercial samples, resembling 18 different brands in Lebanon, were compared with the AR and Ab value of pure carob molasses (0.04 ± 0.01 and 0.90 ± 0.09487 , respectively) 10 and 16 samples, respectively, were found to be significantly different. These findings show high level of adulterations of carob molasses in the Lebanese market and thus need to be studied.

Keywords: Carob permeate; Adulteration; Glucose syrup; Sucrose; Angle of rotation; Wavelength λ ;

Abbreviations: Absorbance (Ab); Angle of rotation (AR); Degree of adulteration (DA).

1. Introduction

Molasses is a concentrated fruit juice produced from various fruits by boiling up to 70% to 80% soluble dry matter content (Akbulut *et al*, 2008). Molasses is the left liquid residue after its preparation by repetitive evaporation, crystallization and centrifugation (Dave, 1998). In general, molasses is produced from raw materials including grape, watermelon, apple, carob,

and sugar beet. Nowadays, in addition to the above mentioned raw materials, apricot and date have been used for the production of molasses in the industrial scale (J. and H.S. Ramaswamy. 2006). Molasses is a liquid food where its rheological characteristics, chemical and physical properties are important for the production process (Karaman and Kayacier 2011). Molasses, also known as “pekmez” in Turkey, is a traditional food product commonly consumed as a breakfast in Turkey and other Middle Eastern countries such as Cyprus and Egypt. Unfortunately little data have been available in Lebanon to estimate the local production, import of raw materials and/or export of finished products.

According to a survey done in Lebanon carob molasses producers stated that the most two products used in adulterations are Sugar (Sucrose) and glucose syrup (Haddara *et al*, 2013).

It is known that most molasses ingredients easily pass into the blood stream without digestion due to its high content in monosaccharide (e.g. glucose) (Akbulut *et al*, 2008). The operations of molasses production vary depending on the origin of fruits; however, the basic process is a concentration of fruit juice or aqueous extract up to certain brix value (Yogurtçu & Kamış, 2006). Brix is an indicator of specific gravity; it is a measure of the total soluble solids (Harrill, 1998). However, brix value does not reveal if these solids are from carob or other sources.

Adulterations, if not controlled, are a major problem in any food product and carob molasses falls in the same category. The main problems with carob molasses adulteration is the dilution of the functional properties with high antioxidant activity (Tounsi, et al.2017.) in addition to the fact of impeachment of the consumer trust. Based on local producers the two main materials used from adulteration are sucrose and glucose syrup.

Nowadays more stringent control on adulterations in processed food is required and stressed upon by the leaflet Food trade and standards published by FAO and WTO in 2017. Methods to detect adulteration such as HPLC (Hurst et al, 2014) are usually expensive and need highly trained personnel to conduct. Thus there is a need to develop a fast easy to conduct and low running cost methods to detect adulteration or at least to reduce the number of samples sent to further detections and thus reduction of costs. The aim of this study is to evaluate a fast low cost method and develop an accurate procedure to detect adulteration using sugar and glucose syrup based on polarimetry and spectrophotometry characteristics of carob molasses.

2. Materials and Methods

2.1. Polarimeter

The polarimeter used in the experiment is Bellingham and Stanley LTD. ADP410 manufactured on January 2007 in United Kingdom. It is an optical instrument with Polarimeter tube 37-251. The polarimeter is used to measure the angle of rotation (AR) of optically active samples.

A 10.0°B of laboratory prepared carob molasses was obtained on RFM700 Refractometer (Bellingham and Stanley LTD. United kingdom) by diluting specific weight of carob with specific weight of distilled water. This weight was calculated by Pearson Square Rule where the initial Brix of carob molasses is previously known. The same calculation was done for 10.0°B glucose syrup and 10°B sucrose. The goal was to see if the adulteration with either glucose syrup or sucrose can be detected. The weight of 0.1°B carob molasses mixed with 0.1°B glucose syrup were (g: g): 20:0, 19.8:0.2, 19.6:0.4, 19.4:0.6, 19.2: 0.8, 19:1, 18:2, 17:3, 16:4, 15:5, 10:10 respectively. Same weight is done for 0.1°B carob molasses mixed with 0.1°B sucrose. The collected carob molasses were checked for their values to be compared by the carob molasses done in the lab without any additive.

Carob Molasses solutions of 0.1°Brix were prepared using distilled water. In this experiment, there is measuring of optical density for: non-adulterated carob molasses samples, commercial samples and non-adulterated carob molasses samples mixed with different levels of sucrose and glucose syrup to establish a baseline in order to detect possible adulteration of commercial samples with either glucose syrup or sucrose by comparing the optical density and angle of rotation.

2.2. Spectrophotometer

Optizen 3220UV/Visible Spectrophotometer (Rose Scientific Ltd., Edmonton, Alberta, Canada) measures the amount of light that a sample absorbs. The transmittance and absorbance are measured for a sample. The same samples used for the polarimeter were used here. A spectrophotometer measures absorbance (A_b) of samples at a wide range of wavelengths (190 nm to 1100 nm) as to check materials detected by different wave lengths and adulteration can be captured. According to the coefficient of determination of the trend-line and the consistency of the A_b data the wavelength $\lambda=302$ was found best fit for this study.

The annotation for syrups studied for adulteration using Glucose was G0: pure non-adulterated carob molasses, G1: 5% glucose syrup + 95% G0, G2: 10% glucose syrup + 90%

G0, G3: 15% glucose syrup + 85% G0, G4: 20% glucose syrup + 80% G0, G5: 25% glucose syrup + 75% G0, G6: 30% glucose syrup + 70% G0, G7: 35% glucose syrup + 65% G0, G8: 40% glucose syrup + 60% G0, G9: 45% glucose syrup + 55% G0, G10: 50% glucose syrup + 50% G0, G100: pure glucose syrup.

Furthermore, those used to study the degree of adulteration (DA) using sucrose was annotated as S0: pure non-adulterated carob molasses, S1: 5% sucrose + 95% S0, S2: 10% sucrose + 90% S0, S3: 15% sucrose + 85% S0, S4: 20% sucrose + 80% S0, S5: 25% sucrose + 75% S0, S6: 30% sucrose + 70% S0, S7: 35% sucrose + 65% S0, S8: 40% sucrose + 60% S0, S9: 45% sucrose + 55% S0, S10: 50% sucrose + 50% S0, S100: pure sucrose.

2.3. Statistical Analysis

All tests and analyses were run triplicate. Quantitative presented data were Mean \pm SEM. One way analysis of variance consisted of univariate and bivariate analysis and Least Significance Difference test (LSD) by General Linear Model (GLM) (high significance at $p < 0.01$ and significance at $p < 0.05$) was used to separate the means difference results, and this was performed using SPSS (Statistical Package for the Social Sciences, version 17.0) program. Bivariate correlations were used and results were obtained as Pearson's correlation coefficients (two tailed). Microsoft Excel 2007 was also used to calculate percentages, minimum and maximum values.

3. Results

3.1. Optical density

Although the coefficient of determination was relatively high for the equation describing the optical density values of sucrose and glucose syrup adulterated samples relation with the DA, 0.9141 and 0.824, there was no significant differences detected between the optical density at different levels of adulteration of both sucrose and glucose and the optical density of pure carob molasses (Table1, 2)

3.2. Angle of rotation

In our attempt to find a fast, easy, applicable, and inexpensive method to detect adulteration, the AR was measured for the simulated carob molasses and for the commercial ones. For that purpose, the angle of rotation of pure unadulterated carob molasses, the simulated ones, was measured. The AR of the simulated pure carob molasses had a mean of 0.04 ± 0.01 . (Table1, 2) The commercial carob molasses had a mean of 0.0361 ± 0.0203 (table 3). Furthermore, it was found that 10 out 18 AR values of commercial carob molasses differ significantly from that of the pure one (table 3).

Comparing AR value of pure carob molasses and those of adulterated ones the difference started being significantly detected at 15% DA using glucose syrup and 35% DA using sucrose (Table1, 2). Concerning the equations describing the relationship between AR and DA using Glucose is described in equation 1 with coefficient of determination (R^2) equal to 0.9961 while the relationship between AR and DA using sucrose is described in equation 2 with an R^2 equals to 0.9758.

Equation 1: $AR = 0.0024DA + 0.0406$

Equation 2: $AR = 0.001DA + 0.0382$

3.3. Wavelength of Carob Molasses, Glucose Syrup and Sucrose

For the same reasons of using the polarimeter and to find another way to detect adulterations, sucrose and glucose syrup, samples of carob molasses were analyzed using UV- Visible Spectrophotometer. A wide range of wavelength (190 nm to 1100 nm) was used in an attempt to find a wavelength that would provide better detection and differentiation for adulteration. Furthermore, commercial, simulated pure and adulterated carob molasses were included in this study. After collecting the data for each wavelength R^2 and slope for the different Ab versus DA were calculated. First, the wavelengths were classified according to different coefficients of determination. The mean of Ab at the chosen wavelength $\lambda=302$ was used to compare the pure carob molasses (0.90 ± 0.09487), with carob molasses samples possessing different DA using glucose syrup and sucrose. Concerning adulteration using Glucose the Ab values started being significantly different from the Ab of the pure carob molasses at the 5% DA (Table 4). While the Ab of sucrose adulterated carob molasses started being significantly different starting at 30% DA (Table 5). Furthermore, it was found that 16 out 18 AR values of commercial carob molasses differ significantly from that of the pure one (Table 6). Concerning the equations describing the relationship between Ab and DA using Glucose is described in equation3 with coefficient of determination (R^2) equal to 0.9958 while the relationship between AR and DA using sucrose is described in equation4 with an R^2 equals to 0.9916.

Equation 3: $Ab = - 0.005DA + 0.9036$

Equation 4: $Ab = - 0.0045DA + 0.8871$

4. Discussion and Conclusion

The optical density was found not fit for the purpose of this study. The AR showed high potential in this matter. Angle of rotation values did not differ significantly at 5% and 10% DA using glucose syrup as an adulteration agent from that of pure carob molasses. In addition

to those findings values of AR observed at 5%, 10%, 15%, 20%, 25% and 30% DA using sucrose as an adulteration agent also did not differ significantly. Thus using this method, sucrose as an adulteration agent needs higher DA to be detected compared to glucose syrup as adulteration agent. This is in accordance the findings that the slope of the AR values of Glucose syrup curve versus those DA was steeper than that of the slope of AR values and DA of the sucrose one, 0.0128 and 0.0051 respectively. Comparing the angle of rotation of the simulated carob molasses and those of the commercial ones, ten out of eighteen samples had no significant difference, and the rest did differ significantly.

Measuring absorbance at $\lambda:302$ nm the Ab values did differ significantly from those of pure carb molasses starting from 5% and above DA with glucose syrup as a fraud agent. The Ab values, however, of Ab started showing a significant difference starting at 30% DA when sucrose was the agent used for adulteration. Same as the polarimetry the sucrose DA can be detected at higher values than those of glucose syrup DA. Also similar to polarimetry the slope of Ab values versus DA for glucose syrup was higher in absolute vale that that of sucrose (-0.005, -0.0045 respectively). However it was a negative slope compared to a positive slope in the results recognized in the lines explaining the relationship of the AR versus DA. This also might explain why the DA using glucose syrup can be detected at a lower level than that of sucrose. Comparing the angle of rotation of the simulated carob molasses and those of the commercial ones, sixteen out of eighteen samples had no significant difference, and the rest did differ significantly.

Furthermore, in this study the Ab seems more suitable for the detection of DA for both sucrose and glucose syrup. This might be due to the fact that polarimeter gives AR only up to two decimals while the Ab values are much more. These findings, although there is a need of more validation, indicate a serious adulteration problem in the Lebanese market. This has to be taken into consideration while conducting any feasibility study since it would affect the competitive advantage of trustworthy carob molasses producers.

This study showed that simple, good established, and easy to conduct methods still have a great potential in the food industry. The polarimetry and spectrophotomerty show high potential if used to examine authenticity of commercial carob molasses. Measuring the absorbance at $\lambda:302$ nm shows higher potential than that of angle of rotation. Although the results should be treated with caution, the 55 to 88 % of the commercial samples being significantly different from the pure sample is very alarming and necessities further research

in the direction of this study. Especially since carob molasses can be used as an ingredient or cacao replacer in many products such as in the bakery industry.

5. References

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Table 1: Mean angle of rotation and optical density for carob molasses samples adulterated with different glucose syrup % compared to non-adulterated carob molasses samples

Glucose syrup + Carob Molasses Samples ¹	Percent adulteration	Angle of rotation	P value of LSD comparing angle of rotation of adulterated with non-adulterated carob molasses	Optical Density
		(Mean± SEM)		(Mean± SEM)
G0	0%	0.04± 0.01		0.40 ± 0.00
G1	5%	0.05 ± 0.02	0.514	0.47 ± 0.21
G2	10%	0.06 ± 0.02	0.310	0.47 ± 0.21
G3	15%	0.08**± 0.03	0.005	0.40 ± 0.17
G4	20%	0.09**± 0.02	0.000	0.43 ± 0.15
G5	25%	0.10**± 0.02	0.000	0.40 ± 0.17
G6	30%	0.11**± 0.03	0.000	0.37 ± 0.21
G7	35%	0.13**± 0.02	0.000	0.30 ± 0.17
G8	40%	0.15**± 0.01	0.000	0.33 ± 0.15
G9	45%	0.14**± 0.03	0.000	0.27 ± 0.12
G10	50%	0.17**± 0.02	0.000	0.30 ± 0.10
G 100	100%	0.28**± 0.01	0.000	0.00 ± 0.00
R²		0.9736		0.9141
Slope		0.0128		-0.045
Intercept		0.0249		0.04864

¹G0: pure non-adulterated carob molasses
G1: 5% glucose syrup + 95% G0
G2: 10% glucose syrup + 90% G0
G3: 15% glucose syrup + 85% G0

G4: 20% glucose syrup + 80%G0
G5: 25% glucose syrup + 75% G0
G6: 30% glucose syrup + 70% G0
G7: 35% glucose syrup + 65% G0

G8: 40% glucose syrup + 60% G0
G9: 45% glucose syrup + 55% G0
G10: 50% glucose syrup + 50% G0
G100: pure glucose syrup

Table 2: Mean angle of rotation and optical density for carob molasses samples adulterated with different sucrose % compared to non-adulterated carob molasses samples

Sucrose + Carob Molasses Samples ²	Percent adulteration	Angle of rotation	P value of LSD comparing angle of rotation of adulterated with non-adulterated carob molasses	Optical Density
		(Mean ± SEM)		(Mean ± SEM)
S0	0%	0.04±0.01		0.30±0.00
S1	5%	0.05±0.03	0.425	0.47±0.21
S2	10%	0.05±0.03	0.425	0.43±0.23
S3	15%	0.05±0.02	0.942	0.40±0.17
S4	20%	0.05±0.02	0.942	0.37±0.21
S5	25%	0.06±0.02	0.310	0.33±0.15
S6	30%	0.07±0.02	0.083	0.33±0.15
S7	35%	0.07*±0.02	0.037	0.30±0.17
S8	40%	0.08*±0.02	0.015	0.30±0.17
S9	45%	0.09**±0.01	0.001	0.27±0.12
S10	50%	0.09**±0.02	0.000	0.27±0.12
S 100	100%	0.14**±0.01	0.000	0.00±0.00
R²		0.9758		0.438
Slope		0.001		-0.004
Intercept		0.0382		0.8243

² S0: pure non-adulterated carob molasses
S1: 5% sucrose + 95% S0
S2: 10% sucrose + 90% S0
S3: 15% sucrose + 85% S0

S4: 20% sucrose + 80% S0
S5: 25% sucrose + 75% S0
S6: 30% sucrose + 70% S0
S7: 35% sucrose + 65% S0

S8: 40% sucrose + 60% S0
S9: 45% sucrose + 55% S0
S10: 50% sucrose + 50% S0
S100: pure sucrose

Table 3: Mean angle of rotation and optical density for commercial carob molasses samples

Carob Molasses Samples ³	Angle of rotation	P value of LSD comparing angle of rotation of adulterated with Non adulterated Molasses	Optical Density
	(Mean± SEM)		(Mean ± SEM)
C1	0.04±0.00	0.156	0.02±0.06
C2	0.04*±0.00	0.042	0.23±0.06
C3	0.02**±0.00	0.000	0.2±0.00
C4	-0.01**±0.01	0.000	0.6±0.00
C5	0.04±0.00	0.084	0.3±0.00
C6	0.05±0.00	0.620	0.3±0.00
C7	0.04*±0.01	0.020	0.3±0.00
C8	0.05±0.00	0.620	0.2±0.00
C9	0.08**±0.00	0.004	0.3±0.00
C10	0.05±0.00	0.620	0.2±0.00
C11	0.06±0.01	0.174	0.6±0.00
C12	0.03**±0.01	0.003	0.23±0.06
C13	0.02**±0.00	0.000	0.17±0.06
C14	0.01**±0.00	0.000	0.2±0.00
C15	0.05±0.01	0.901	0.63±0.06
C16	0.04±0.01	0.174	0.37±0.06
C17	0.01**±0.00	0.000	0.27±0.06
C21	0.03**±0.00	0.001	0.4±0.00
Mean	0.0361±0.0203		0.3205±0.06
Min	-0.01		0.17
Max	0.08		0.63

³ C: Commercial carob molasses samples

Table 4: Mean absorption at $\lambda = 302$ nm of adulterated carob molasses samples with glucose syrup

Glucose syrup +Carob Molasses Samples ⁴	Percent adulteration	Absorption @ $\lambda : 302$ nm	P value of LSD comparing absorption of adulterated with non - adulterated carob molasses
		(Mean \pm SEM)	
G0	0%	0.90 \pm 0.09487	
G1	5%	0.861667* \pm 0.052691	0.009
G2	10%	0.868667* \pm 0.081082	0.016
G3	15%	0.820667** \pm 0.057361	0.000
G4	20%	0.811667** \pm 0.049095	0.000
G5	25%	0.787667** \pm 0.026502	0.000
G6	30%	0.762** \pm 0.027	0.000
G7	35%	0.725** \pm 0.031575	0.000
G8	40%	0.700667** \pm 0.020744	0.000
G9	45%	0.676333** \pm 0.022942	0.000
G10	50%	0.653667** \pm 0.026312	0.000
G100	100%	0.402333** \pm 0.022301	0.000
R²		0.988178	
Slope		-0.00501	
Intercept		0.904894	

⁴ G0: pure non-adulterated carob molasses
 G1: 5% glucose syrup + 95% G0
 G2: 10% glucose syrup + 90% G0
 G3: 15% glucose syrup + 85% G0

G4: 20% glucose syrup + 80%G0
 G5: 25% glucose syrup + 75% G0
 G6: 30% glucose syrup + 70% G0
 G7: 35% glucose syrup + 65% G0

G8: 40% glucose syrup + 60% G0
 G9: 45% glucose syrup + 55% G0
 G10: 50% glucose syrup + 50% G0
 G100: pure glucose syrup

Table 5: Mean absorption at $\lambda = 302$ nm of adulterated carob molasses samples with sucrose

Sucrose +Carob Molasses Samples ⁵	Percent adulteration	Absorption @ $\lambda : 302$ nm	P value of LSD comparing absorption of adulterated with non - adulterated carob molasses
		(Mean \pm SEM)	
S0	0%	0.90 \pm 0.09487	
S1	5%	0.86933 \pm 0.061232	0.282
S2	10%	0.844 \pm 0.052374	0.651
S3	15%	0.827667 \pm 0.045742	0.961
S4	20%	0.791667 \pm 0.033724	0.402
S5	25%	0.778333 \pm 0.044636	0.244
S6	30%	0.737333* \pm 0.032868	0.031
S7	35%	0.721333* \pm 0.027592	0.011
S8	40%	0.703667** \pm 0.005686	0.003
S9	45%	0.67** \pm 0.027221	0.000
S10	50%	0.647667** \pm 0.027429	0.000
S100	100%	0.448667** \pm 0.113391	0.000
R²		0.996015	
Slope		-0.00491	
Intercept		0.893909	

⁵ S0: pure non-adulterated carob molasses

S4: 20% sucrose + 80% S0

S1: 5% sucrose + 95% S0

S2: 10% sucrose + 90% S0

S3: 15% sucrose + 85% S0

S8: 40% sucrose + 60% S0

S5: 25% sucrose + 75% S0

S6: 30% sucrose + 70% S0

S7: 35% sucrose + 65% S0

S9: 45% sucrose + 55% S0

S10: 50% sucrose + 50% S0

S100: pure sucrose

Table 6: Mean absorption at $\lambda = 302$ nm of commercial carob molasses samples compared to non-adulterated carob molasses samples

Carob Molasses Samples ⁶	Absorption @ $\lambda : 302$ nm	P value of LSD comparing absorption of adulterated with non - adulterated carob molasses
	(Mean \pm SEM)	
C1	0.758** \pm 0.022	.004
C2	0.843 \pm 0.175	.462
C3	0.790* \pm 0.006	.034
C4	0.957* \pm 0.027	.027
C5	0.736** \pm 0.021	.001
C6	0.702** \pm 0.017	.000
C7	0.778* \pm 0.022	.016
C8	0.805 \pm 0.039	.086
C9	0.717** \pm 0.058	.000
C10	0.6968* \pm 0.030	.000
C11	0.735** \pm 0.030	.001
C12	0.716** \pm 0.042	.000
C13	0.639** \pm 0.039	.000
C14	0.677** \pm 0.049	.000
C15	0.745** \pm 0.073	.001
C16	0.977** \pm 0.080	.007
C17	1.019** \pm 0.081	.000
C21	0.676** \pm 0.067	.000

⁶ C: Commercial carob molasses samples