



Impact of Hydrocolloids on Production, Quality Attributes and Nutritional Values of Egyptian Black Mulberry Fruits (*Morus nigra* L.) Leathers



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Abstract

Black mulberry (*Morus nigra* L.) is a popular, nutritional and palatable fruit in Egypt. In this research, trials were carried out to assess the quality of fruit leathers produced from black mulberry fruits under the application of different hydrocolloids (pectin, carboxy methyl cellulose CMC and Xanthan) for improving textural, functional and sensorial characteristics of the obtained leathers. Preliminary experiments proved that use of 1.25% pectin as binding agent produced superior fruit leather regarding rheological, textural, color and organoleptic quality compared with those produced using CMC or Xanthan as thickening agents. Based on these results, experiments were further continued to assess the impact of deseeding black mulberry fruit pulp and application of sun (solar) or oven drying on kinetics of drying process and quality attributes of the obtained leathers. Results showed that most of the drying pattern was in the falling rate drying phase and drying required 13 h and 18 h in oven (55 °C) and solar drying, respectively, Henderson and Pabis as well as Wang - Singh equations were the most suitable model to represent the drying pattern. SEM examination of leather surfaces showed a homogeneous structure and use of 1.25% pectin enhanced the integrity of the leather matrix through gelation and structure binding. Presence of seeds in the leather led to some cracks in the surface structure of the obtained leathers. It could be concluded that black mulberry fruits could be successfully used to produce good quality and nutritional leathers, when using pectin at the level of 1.25%.

Keywords: Black mulberry, Fruit leathers, Drying kinetics, Hydrocolloids Application, Scanning electron microscopy (SEM).

1. Introduction

Mulberry fruit (*Morus nigra* L.) has become a popular fruit nowadays due to its high nutritional value. [1] The mulberry fruit is a member of the Moraceae family and belongs to the genus *Morus* and it possess a wide scope of biochemical activities, such as antioxidant, anti-hyperlipidemia and anti-cancer properties, due to their rich natural phenolic compounds, including phenolic acids, flavanols and anthocyanins. It could be considered as a great source of several vital nutrients, namely calcium, phosphorus, potassium and magnesium. However, different phenotypes have different mineral contents. [1-3]

Egypt is one of the largest countries in Africa and the Middle East producing of berry fruits. Sohag governorate is famous for planting mulberry and using leaves in raising silkworms and making silk textiles. Furthermore, the Egyptian government has also worked to increase the area planted with berries, as it has established a project to cultivate

one million mulberry trees in the Delta and many governorates such as Al wadi Al gadid (New Vally) and the Red sea. [4]

Abouzed et. al. [2] reported that 100 g of fresh Mulberry fruit contains: 32 calories, 90.95g water, 0.67g protein, 0.3g fat, 7.68g carbohydrates, 4.89g sugars and 2.0g water soluble fibers. Also it has, 153mg potassium, 24 mg phosphorus, 16mg calcium, 13mg magnesium, 0.41 mg iron, and 0.14 mg zinc, as well as, 58.8mg vitamin C, 0.386mg niacin, 0.047 mg B6, 24mg Folate, 0.29mg vitamin H and 0.022mg riboflavin. Due to inadequate post-harvest handling facilities, the losses are as high as 22%. To reduce the wastage, the fruit can be preserved in the form of frozen, canned and dried products [5]. It is also processed into various products like Jams, Jellies, Juices etc. Dried fruit rolls, such as Mulberry ones has become a popular dried fruit, especially, for school students due to its delicious taste and nutritional value. Nowadays, many producers and researchers aimed to produce high quality of these

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products with attractive physical sensory properties and high nutritional value. Hydrocolloids can be defined as heterogeneous group of long chain polymers which form viscous dispersions and/or gels when dispersed in water.

Hydrocolloids can be either polysaccharides or proteins. Additionally, hydrocolloids act as health promoters by reducing the risk of cardiovascular diseases, reducing the risk of obesity, regulating glycemic response and maintaining colonic health.] Food hydrocolloids are used in fruit snack formulations to create a new texture, increase stability for their water-holding capacity, improve consistence and have an impact on flavor release and other structural and sensory properties in the respective product. [6-7]

Black mulberry is a fruit which have a lot of glucose, fructose, sucrose and phenolic acids. Use of suitable drying methods and hydrocolloidal serving binders is a common issue that is beneficial to protect nutrients material of these fruits, Ishwarya et. al. [8] found that pectin could provide a soft and shiny gel. It restricted loss of nutrient and volatile materials during storage and transport of product. [9-13]

Also, contamination of product by microorganisms could be controlled by pectin hydrocolloid material. There is a significant interest in the industry to improve the energy efficiency in dehydration to maintain its profitability, competitiveness and sustainability due to the prospect of a continuously increasing trend in fuel prices and the need for eco-friendly processes to mitigate environmental impact coupled with the rising consumer demand for high quality products.

Therefore, this research is focused on studying the effect of different hydrocolloids (pectin - Carboxymethyl Cellulose (CMC) and xanthan) on the sensory properties, nutritional value, Physical properties, color, microstructure and microbial shelf Life of Egyptian black mulberry leather.

2. Material and Methods

2.1. Materials

2.1.1. Raw Materials

Black mulberry fruits (*Morus nigra*) were obtained from local market, Menoufia Governorate, Egypt on the same day of harvesting and kept under cooling until processing. The total solids of the obtained fruits were 14.84% in average.

2.1.2. Chemicals and other ingredients

Pectin, Xanthan, Carboxymethyl Cellulose (CMC), citric acid, glycerol and Sodium Metabisulfite of analytical grade were obtained from El-Nasr Pharmaceutical Chemicals Co., Egypt. (ADWIC). Sugar was purchased from local market, Egypt.

2.2. Methods

2.2.1. Preparation of Black Mulberry Leather

Fruits of black mulberry were selected according to uniformity of shape and color, washed with tap water to remove surface dirt and used for analysis and processing.

Fruits were squeezed with household blender (moulinex France). Half of obtained puree was de-seeded by sieving using a cheese cloth to produce smooth leather. (Black Mulberry Fruit Leathers), while the other half of obtained puree was passed through strainer to remove clumps and was used to produce black Mulberry leather. Eight formulations were designed and presented in Table (1). Each formula was cooked in water bath for 20 min using heater to reach temperature up to 70 °c and spread uniformly on stainless steel tray with a layer thickness of 3 mm. The heat treatment is essential to avoid enzymatic browning, to soften the tissue, to reduce microbial load and to allow polymers to be dissolved and distributed before drying. Glycerol was used for easy scrapping off the leather after drying. The samples were dried from one side (top surface). The initial moisture content of the puree samples mixture was 78.75% (wet basis). The drying regimes were as follows:

For Hot air oven drying (O), the puree was spread as before and dried in preheated oven (Venti cell forced air convection oven USA) at 55°C ± 2°C until the moisture content of 15-20% has been achieved. The dried leather was removed from the tray and cut into the desired size or rolled. Various hydrocolloids were investigated such as pectin, CMC and xanthan as given in Table (1).

For Sun (Solar) drying (S), the puree mixture was spread, as before, and was dried on a tray at a temperature range of 35 to 64 °C ± 2 over the day for 3 days in solar dryer. Moisture loss was recorded at 1h intervals until the final stage of drying and then proceeded as before. Final products were cut into uniform square shapes (approx. 2.5 cm × 2.5 cm) using a sharp knife. Low Density Poly-ethylene (LDPE) plastic was used for packing and then kept under cooling for palatability assay, color evaluation, physicochemical properties, microbial assay and microstructure up to 9 months.

2.2.2. Rheological behavior of black mulberry puree samples (de-seeded and not de-seeded)

Rheological properties of mulberry puree obtained by blending were studied using Brookfield Digital Rheometer, model HA DVIII Ultra (Brookfield Engineering Laboratories INC.), with spindle No. SC4-21.

Table 1: Black Mulberry Leather formulations

Ingredients	de-seeded formulations			Not de-seeded formulations		
	P	C	X	PS	CS	XS
Pectin%	1.25	-	-	1.25	-	-
Carboxymethyl Cellulose (CMC)%		0.5	-	-	0.5	-
Xanthan%	-	-	1.25	-	-	1.25
Sugar (g)	60	60	60	60	60	60
Black Mulberry(g)	1000	1000	1000	1000	1000	1000
citric acid (g)	2	2	2	2	2	2
Sodium Metabisulfite (g)	0.2	0.2	0.2	0.2	0.2	0.2

P= Pectin formula, C= CMC-formula and X= Xanthan formula

PS= Pectin formula with seeds, CS= CMC-formula with seeds and XS= Xanthan formula with seeds

Blends were placed in a small sample adapter, the SC4-21 spindle was selected for the sample measurement according to Sengül et. al. [14] Shear rate /shear stress data were analyzed according to the power law model: [15-18]

$$\tau = K * \gamma^n \tag{1}$$

The values of apparent viscosity (η_a) were calculated as following:

$$\eta_a = K * \gamma^{n-1} \tag{2}$$

Where: γ is the shear rate (S^{-1}), τ is the shear stress (Pa), K is the consistency coefficient (Pa. s^n), n is the flow index (-), and η_a is the apparent viscosity (m Pa. s)

2.2.3. Palatability assay

The palatability assay was carried out to choose the best acceptable treatment (Formula) for preparation of mulberry leather. For this purpose, a primary drying test was complete for different puree mix samples and the obtained dried leathers were subjected to organoleptic analysis by a panel test of 10 staff members at Food Technology Institute, Agriculture Research Center, Giza, Egypt. The panelists were asked to evaluate the sensory scores (taste, color, texture and overall acceptability) on a scale of 1 to 10 for the fresh dried mulberry leather.

2.2.4. Evaluation of drying curves, rates and modeling

2.2.4.1. Drying curves

Drying curves of mulberry fruit leather were obtained as the change in moisture content during the period of drying. Moisture content (MR) was expressed as the ratio of water content (M H₂O) divided through the constant weight of dry matter M_{DM} as follows:

$$MR = \frac{M_{H_2O}}{M_{DM}} \dots\dots\dots (g \text{ H}_2\text{O}/ g \text{ DM}) \tag{3}$$

During drying process, the weight of dry matter M_{DM} remains constant, while M H₂O changes (decreases) with the progress of drying time. Values of

obtained MR versus time of drying were plotted to give the characteristic drying curve

2.2.4.2. Drying Rate

Drying rate expresses the amount of removed water per one unit of drying time and has the expression:

$$\text{Drying rate} = \frac{g \text{ mH}_2\text{O}}{g \text{ DM.h}} \tag{4}$$

it is important for describing the pattern of water removal during the different phases of drying and indicates the mode of moisture movement inside the drying food layer.

Modelling drying curves

The course of moisture change during drying gives no straight line, but curved course with different phases. To simulate the drying curves of foods, different mathematical models have been developed based on the degree of difficulty of moisture movement inside the food matrix during drying process. In this work, three different models were applied for drying mulberry leather as follows: [19, 20]

Henderson and Pabis:

$$MR = a. e^{-k.t} \tag{5}$$

1- **Page:** $MR = a. e^{-k.t^n}$ tag(6)

2- **Wang and Singh:** $MR = at^2 + bt + c$ tag(7)

Where:

MR = Moisture content (g H₂O / g DM),

t = Drying time (hr.) and

a, b, c, n and k are constants. (K-value represents the overall drying rate).

The validity of each model was judged to present the drying data, according to the following statistical parameters: [19, 20]

Correlation coefficient (R²), sum square of error (SSE), root mean square of error (RMSE), X² Parameter and relative percentage of deviation (P%), Calculated as follows: [21, 22]

$$R^2 = 1 - \frac{\sum_{i=1}^N (MR_{pred} - MR_{exp})^2}{\sum_{i=1}^N (MR_{pred} - MR_{exp})^2} \tag{8}$$

$$P\% = \frac{100}{N} \left| \frac{MR_{exp} - MR_{pre}}{MR_{pre}} \right| \quad (9)$$

$$SSE = \sum_{i=0}^N (MR_{exp} - MR_{pre})^2 \quad (10)$$

$$X^2 = \frac{\sum_{i=1}^N (MR_{exp} - MR_{pre})^2}{N} \quad (11)$$

$$RMSE = \left(\frac{1}{N} \sum_{i=1}^{N-m} (MR_{exp} - MR_{pre})^2 \right)^{0.5} \quad (12)$$

Where: MR_{exp} and MR_{pred} are experimental and model calculated moisture content (g H₂O/g DM)

N= Number of replicates (observations)

m = Number of constants in each model

The parameters were calculated by using statistical package (SPSS).

2.2.5. Chemical assay

Protein (N x 6.25 Kjeldahl method), fat (hexane solvent, Soxhlet apparatus), fiber and ash were determined according to the method recommended by AOAC. [23] Total carbohydrates were estimated by difference. Total and reducing sugars were determined by the method of Somogy as adapted by Nelson. [24] Total Anthocyanin content was determined according to Giusti et. al. [25] DPPH free radical scavenging activity was carried out as described by Barros et. al. [26] Total flavonoid according to AOAC [23] while total phenolics were measured as described by Velioglu et. al. [27]

2.2.6. HPLC analysis of individual sugars

For the hydrolysis of sugars, 2g of samples were weighed, soaked in 8ml of bi-distilled water, then homogenized (Ultra-Turrax, Ika Labor Technik, Germany) and were extracted (hydrolyzed) for 30 minutes at 20°C under continuous shaken as described by Mikulic-Petkovsek et. al. [28] The extracts were then centrifuged and filtered through 0.2 µm filters (chromafilPP/ MVA-20/25, Masherey - Nagel, Dueren, Germany) into glass vials. Chromatographic analysis was performed using HPLC system of Finnigan surveyor (Thermo Fischer Scientific, CA, USA).

2.2.7. Physical assay

Moisture content was determined according to AOAC. [23]. Total soluble solids were determined using refractometer (Atago co, Ltd, Japan) at 20°C. water activity of samples was measured at 25°C by the AOAC 978. [29] hygrometric method [13] using an Aqualab water activity meter (Novasina AG, Switzerland). Texture analysis was performed using texture analyzer (single arm texture analyzer TA-XT Plus, Stable Micro Systems, Surrey, UK) with a load cell of 2 kg weight. A force versus time curve was recorded for a tensile test until cutting at a displacement speed of 10 mm/min. In-built software of the texture analyzer was used for analyzing the data generated. Scanning electron microscopy (SEM) was used to visualize the microstructure of the rolls, as described by Nasr et. al. [30] Color analysis of

black mulberry leather was carried out on the outer surface of the dried samples using a Chroma-meter device (Konica, Minolta, Japan) according to McGuire [31]. Lightness (L: 0-100), redness (a: +60 to -60) and yellowness (b: +60 to -60) values were directly obtained and used to estimate color saturation (C: $\sqrt{a^2 + b^2}$) and color type (Hue: $\tan^{-1} \frac{b}{a}$) as well as incidence of browning.

2.2.8. Microbiological analysis

Total bacterial count, Yeast and Mold were carried out during different storage periods according to AOAC 966.23. [32]

2.2.9. Statistical analysis

The statistical analysis was performed using SPSS One-Way ANOVA, version22 (IBM Corp.) released in 2013. Data were treated as a complete randomization design according to Steel et. al. [33] Multiple comparisons were carried out applying the Duncan test. The significance level was $P < 0.05$.

3. Results and Discussions

3.1. Rheological behavior of black mulberry puree samples (de-seeded and not de-seeded)

Table (2) shows the results of rheological properties of mulberry fruit puree as analyzed by equations 1 and 2 for puree prepared from de-seeded and non-de-seeded blends (mixtures). As seen, blends showed non-Newtonian behavior since n-values were less than unity with blends containing xanthan gum as extremely non-Newtonian fluid with the lowest n-values of 0.175 and 0.1788. Deseeding of mulberry puree significantly reduced the values of consistency coefficient by 5 to 10 folds. Addition of xanthan as thickening agent extremely increased the viscosity values of all blends (mixture), with K-value of (29.58 pa.sⁿ) for the non-seeded mixture. On the other side, use of pectin and CMC as thickening agent resulted in moderate raise in K- values to the range of (12.03 to 17.44 pa.sⁿ) compared with that of the puree mixture without thickening agent (6.16 pa.sⁿ). For a fundamental comparison between the different mulberry puree mixtures, the values of apparent viscosity (µa) at shear rate value of 5 s⁻¹ and the results were included in Table (2). These values revealed that not only the deseeding process reduced the viscosity values, but also the type of the used thickening agent. The obtained results agree with those of Sengül et. al. and Antonio et. al.. [14, 34] They reported non-Newtonian and pseudo-plastic behavior of mulberry and blue berry purees as well as consistency coefficient values in the range of 13 to 17 pa.sⁿ, which confirm the results obtained in the present work.

3.2. Some Physical properties of dried black mulberry leather

As mentioned before, black mulberry puree was obtained and different thickening agent (pectin, CMC and xanthan) were added and the mixtures were dried and subjected to some physical and sensory examinations in order to get the most acceptable mixture (the most accepted thickening agent) and results of physical tests are given in Table (3). The dried samples were examined for their moisture content, total solids, water activity, thickness, hardness, tensile force as the important quality parameters determining their acceptability by consumers. As seen, the total solids content of the tested leather samples ranged between 82.4 % to 83.33% and the moisture content 16.67% to 17.60%. Sun (solar) dried samples showed final moisture content slightly higher than those ovens dried. No significant (remarkable) differences in total solids and moisture content were found related to the type of the added hydrocolloid (pectin, CMC, xanthan). Values of water activity (a_w – values) were in harmony with the final moisture content of the tested samples. All recorded a_w -values were lower than 0.6, being in the safe region for possible mold and yeast growth, but not safe for enzymatic and oxidative activity (which requires a_w - values lower than 0.3). Generally, water activity values of sun (solar) dried samples were slightly higher than those ovens dried and a_w -values of xanthan fortified samples were higher than those recorded for pectin or CMC fortified ones. Thickness of the obtained leathers were in the range of 0.87 mm to 1.23 mm with the sun (solar) dried samples have higher thickness than those ovens dried, may be due to the relativity higher final moisture content of the first. Hardness values of the obtained leather ranged between 324.23 to 739.67 gf.

Table 2: Rheological parameter of mulberry puree prepared with different thickening agents

Sample type		Consistency coefficient (K) Pa. s ⁿ	Flow behavior index (n)	R ²	Apparent viscosity (η_a) mPa. s
Puree with seed	Control Puree	6.018±0.183 ^d	0.332±0.005 ^{de}	0.987±0.009 ^{ab}	2056.75±79 ^d
	puree+ Pectin	11.811±0.351 ^c	0.360±0.018 ^c	0.966±0.019 ^b	4230.98±246 ^c
	puree+ CMC	17.482±0.487 ^b	0.313±0.006 ^c	0.995±0.003 ^a	5788.44±100 ^b
	puree+ Xanthan	29.565±0.521 ^a	0.175±0.006 ^f	0.999±0.00 ^a	7851.96±218 ^a
Puree without seeds	Control Puree	0.566±0.055 ^f	0.342±0.006 ^{cd}	0.992±0.003 ^a	913.56±737 ^{ef}
	puree+ Pectin	1.233±0.058 ^f	0.394±0.004 ^b	0.964±0.003 ^b	465.33±25 ^f
	puree+ CMC	2.536±0.124 ^e	0.486±0.006 ^a	0.988±0.001 ^{ab}	1111.87±66 ^{ef}
	puree+ Xanthan	5.447±0.115 ^d	0.176±0.007 ^f	0.987±0.002 ^{ab}	1448.97±47 ^{de}

Note: Means within a column with the same superscript are not significantly different (P < 0.05)

The highest darkness level was recorded for mulberry leather samples prepared without use of hydrocolloide polymer (L= 16.29), while the leather samples prepared by incorporation of 1.25% pectin were less dark, more brightness and gloss with L-values of 21.66 and 24.57 for oven and sun (solar) dried samples, respectively. leather samples containing xanthan or CMC were darker than those containing pectin with L-values lower than those containing pectin. Due to the darkness of black mulberry leather samples, all values of a-axis and b-

pectin containing samples showed higher hardness values than those of CMC and xanthan, may be due to the ability of pectin to form a gel net under presence of sugar, citric acid and heat. On other side, oven dried samples showed higher hardness values than those sun (solar) dried ones.

Also, the presence of seeds reduced the hardness values of the obtained leather sheets. Hardness values comply with a_w -values of the obtained leather, where samples with lower a_w values showed higher hardness levels. The tensile force of the obtained leather samples showed values in the range 12.63 to 17.5 N, where xanthan containing samples showed the highest values (16.77 to 17.50 N) and pectin containing ones the lowest values (12.63 to 13.57 N). Leather samples containing seeds showed tensile force lower than those strained before drying. Increasing thickness of leather sheets resulted in higher tensile force values. These results agree with those of Karki et. al. [35] who reported moisture content 21.5 to 23.9 %, Tensile force 15.5 to 22.1 N and hardness value of 345 to 759 g for blueberry fruit leather.

3.3. Color parameters of primary samples of black mulberry leather

Although the appearance of all black mulberry leather samples is black, but some differences could be obtained due to the type and concentration of used hydrochloride polymer and drying method. Table (4) includes the Hunter Lab color parameters of the obtained leather samples. The Lightness (L-values) of all samples (control sample and those containing pectin or xanthan or CMC) were low in the range of 16.29 to 24.57. Low values on the lightness scale (0-100) means that all leather samples are dark colored with different intensity.

axis of color coordinate were located at the center of color axis (gray zone) with very low color saturation (C-values color scale and ranging between 0.85 to 1.54). Also, redness color shade (a-values) was close to zero (0.36) or showed negative sign (-0.7 to -1.46) indicating a light greenish color shade for the leather samples. On other side, yellowness parameter (b- values) were all in the gray center zone of the color coordinate and showed low values between 0.43 to 0.85. Decisively important is the color type (Hue values) of the tested leather samples. Pectin

containing samples (oven or solar dried) showed the highest dark color type on the coordinates sphere 159.24° and 160.74° , while the xanthan or CMC – containing samples showed lower H° -values (145.08° to 157.73°). Presence of polymers (hydrocolloids) in the leather formula reduced the incidence of browning reactions during drying. Browning index (BI) values were reduced from 7.01 for control sample to the level of 0.2 to 2.30 for polymers containing samples. Based on their results, pectin containing samples showed superior brightness and higher Hue $^\circ$ -Values compared with those containing xanthan or CMC.

3.4. Sensory evaluation of prepared mulberry leather samples:

Fig (1) shows the scores of the sensory attributes (taste, color, texture and overall acceptability) rec-

Table 3: Physical characteristics of the Black Mulberry leather

Samples	Total solid %	Water activity (a_w)	Texture characteristics		
			Hardness (g)	Tensile Force (N)	Thickness (mm)
Po	83.07±0.29 ^{bcd}	0.46±0.00 ^d	739.67±16.38a	13.57±0.12f	0.87±0.33 ^b
Ps	82.40±0.53 ^a	0.47±0.01 ^{cd}	692.00±9.07 ^b	13.00±0.12 ^g	1.10±0.00 ^{ab}
Co	83.33±0.23 ^a	0.57±0.01 ^a	481.00±13.05 ^g	14.63±0.03 ^{cd}	1.13±0.03 ^{ab}
Cs	82.73±0.58 ^a	0.58±0.00 ^a	466.00±6.43 ^g	14.30±0.10 ^{de}	1.13±0.03 ^{ab}
Xo	82.50±0.36 ^a	0.53±0.00 ^b	595.00±4.51 ^d	17.50±0.06 ^a	1.20±0.00 ^a
Xs	82.40±0.45 ^a	0.54±0.00 ^b	555.33±5.21 ^e	17.13±0.12 ^b	1.20±0.00 ^a
POS	83.10±0.21 ^a	0.47±0.00 ^c	643.67±2.40 ^c	12.93±0.07 ^{gh}	1.13±0.03 ^{ab}
PSS	82.73±0.44 ^a	0.48±0.01 ^c	598.00±3.79 ^d	12.63±0.20 ^h	1.20±0.00 ^a
COS	82.57±1.93 ^a	0.53±0.01 ^b	369.00±16.86 ^h	14.20±0.25e	1.20±0.00 ^a
CSS	82.37±1.07 ^a	0.54±0.00 ^b	324.33±11.10 ⁱ	14.83±0.12c	1.23±0.03 ^a
XOS	83.30±0.35 ^a	0.53±0.00 ^b	533.33±5.78e ^f	16.90±0.06 ^b	1.23±0.00 ^a
XSS	82.60±0.31 ^a	0.54±0.00 ^b	517.67±23.38 ⁱ	16.77±0.03 ^b	1.33±0.03 ^a

P= pectin, C= Carboxymethyl Cellulose (CMC), X= Xanthan, O= oven dried, S= sun dried, S= with seeds.

Note: Means within a column with the same superscript are not significantly different ($P < 0.05$)

Table 4 Tristimulus lab color parameters of dried black mulberry leather

Treatments	L*	a*	b*	C	H $^\circ$	BI
Control	16.29±0.51 ^d	0.36±0.07 ^a	0.85±0.24 ^a	0.95±0.21 ^c	67±0.87 ^{ab}	7.01±1.77 ^a
PO	21.66±0.25 ^{bc}	-1.24±0.03 ^d	0.47±0.01 ^b	1.33±0.03 ^{ab}	159.24±0.15 ^b	2.04±0.05 ^{bc}
XO	20.79±0.28 ^b	-0.97±0.02 ^c	0.54±0.01 ^b	1.11±0.02 ^{bc}	150.90±0.07 ^b	0.85±0.09 ^b
CO	16.36±0.70 ^d	-0.70±0.08 ^b	0.49±0.04 ^b	0.85±0.07 ^c	145.08±0.20 ^b	0.20±0.39 ^b
PS	24.57±1.61 ^a	-1.46±0.06 ^c	0.51±0.02 ^b	1.54±0.05 ^a	160.74±6.82 ^a	2.30±0.21 ^c
XS	19.65±0.73 ^c	-0.97±0.06 ^c	0.49±0.05 ^b	1.09±0.07 ^{bc}	153.2±0.21 ^b	1.14±0.24 ^{bc}
CS	22.09±0.38 ^b	-1.05±0.06 ^{de}	0.43±0.02 ^b	1.42±0.06 ^a	157.73±3.17 ^b	2.55±0.24 ^c

Note: Means within a column with the same superscript are not significantly different ($P < 0.05$)

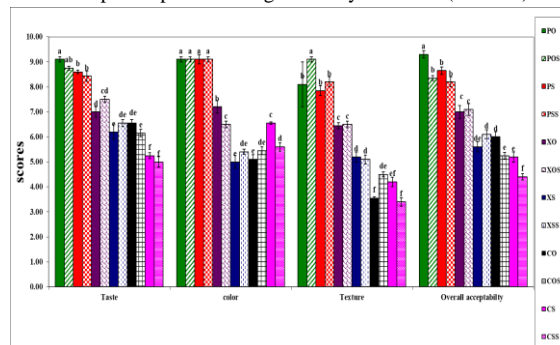


Figure 1: Sensory perception analysis of various formulations of Black mulberry rolls: P= pectin, C= Carboxymethyl Cellulose (CMC), X= Xanthan, O = oven dried, S= sun dried, S= with seeds.

They stated that use of pectin in production of pear and persimmon leather enhanced the quality and sensory attributes of the obtained products.

3.5. Evaluation of drying curves, rates and modeling

3.5.1. Drying Curves of Solar and Oven Dried Mulberry Sheets

Fig (2) shows the drying curves of mulberry sheets containing pectin during sun (solar) and oven drying. Drying curves illustrate the change in moisture ratio MR ($\text{g H}_2\text{O/g DM}$) during the drying time. As observed, all drying curves showed a short constant drying rate phase followed by phases of falling drying rate indicating the complicated moisture transfer from inside the sheet to the surface, where drying takes place. As seen, hot air oven drying was faster (14 h) than that of sun (solar) drying (20 h) over three days of drying. Samples dried by hot air oven reached the safe final moisture content ($\approx 20\%$), while those sun (solar) dried reached higher moisture content (22.5 - 23%) at the end of drying process. The reason could be referred to the difficulty of moisture movement from inside the sheets to the surface at the end of drying period, where the rest moisture is located at the center of the sheet and needs high air temperature to assist the diffusion of water molecule from the core to the surface. Pres-

ence or removal of seeds from the sheet pulp did not significantly affect the final moisture content.

3.5.2. Drying rate

Fig (3) shows the drying rate ($\text{g H}_2\text{O} / \text{g DM. h}$) during the drying process. As seen, the drying rate curves could be divided in three phases. The first phase (first hour) indicates a gradual increase in the leathers temperature and hence a continuous increase in the drying rate from 0 to the assigned highest drying rate. The second phase is that of constant drying rate, which was obvious in the leathers containing seeds than that without seeds (either sun or oven dried). The third phase is that of falling drying rate until the moisture content of the dried leathers reached an equilibrium state with the drying air and the drying rate approaches 0 ($\text{g H}_2\text{O} / \text{g DM. h}$). Constant drying rate phase of sun (solar) dried sheets was $0.5 \text{ g H}_2\text{O} / \text{g DM. h}$ for sheets containing seeds and $0.61 \text{ g H}_2\text{O} / \text{g DM. h}$ for leathers dried without seeds, which reflects the role of seeds in hindering moisture movement. The critical moisture content (CMC), at which, the drying pattern shifts from constant to falling rate was 2.4 and $3.1 \text{ g H}_2\text{O} / \text{g DM}$ respectively for sun (solar) dried with and without seeds, while this parameter could not be observed for leathers dried in oven.

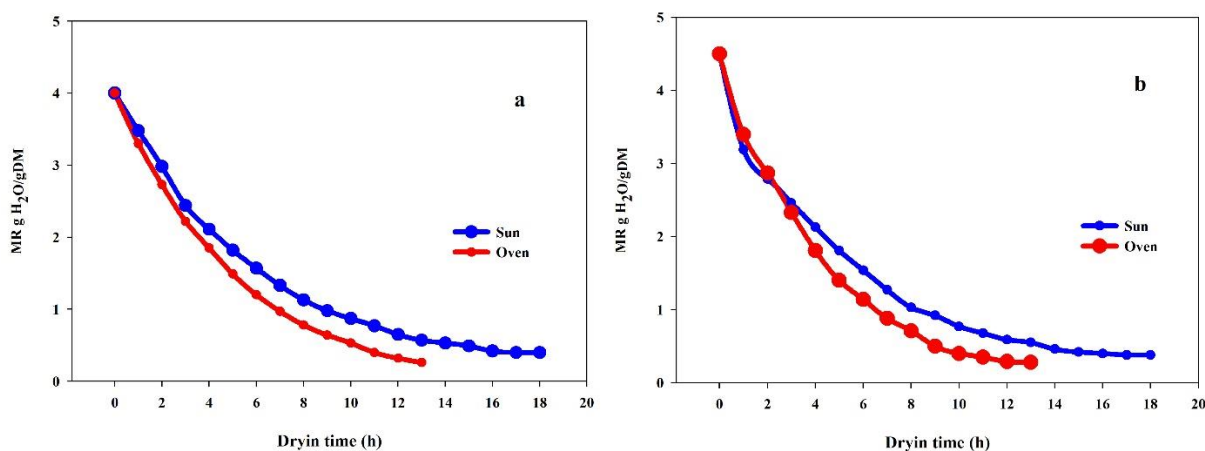


Figure 2: Drying curves of sun (solar) and oven dried mulberry leather (a: without seeds, b: with seeds)

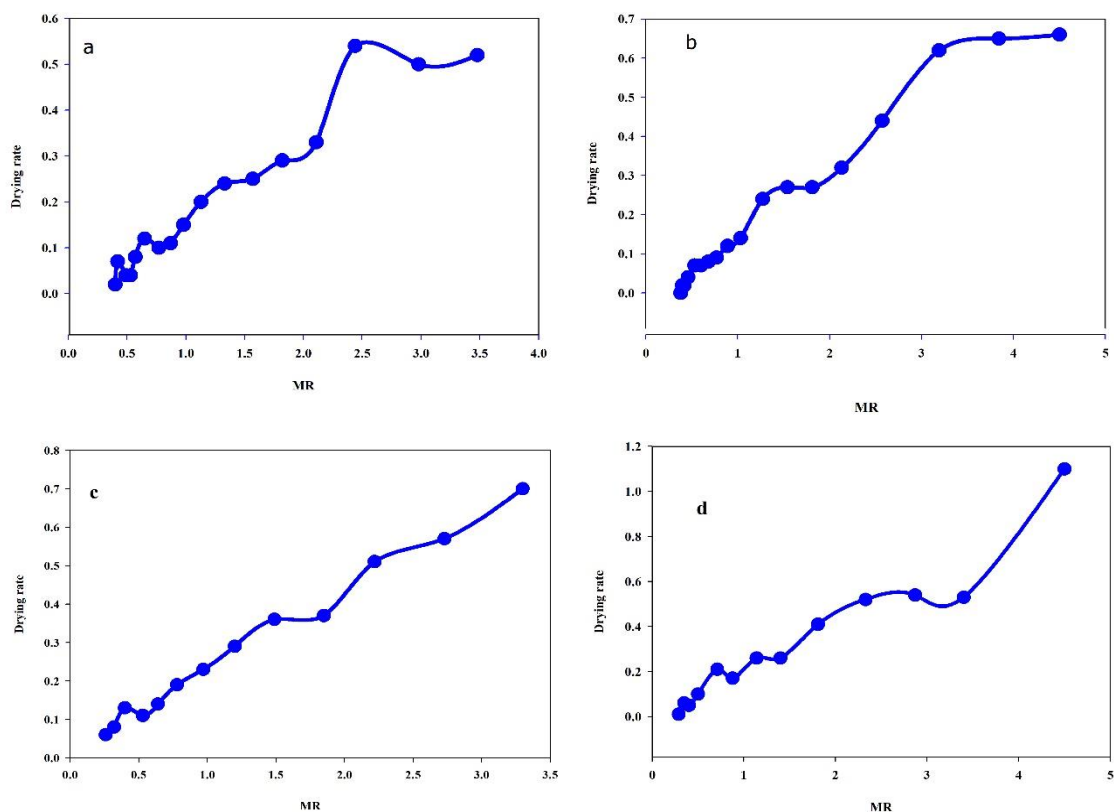


Figure 3: Drying rate g H₂O/g DM. h for black mulberry leather (a =sun with seeds, c = Oven with seeds, b = Sun without seeds and d= Oven without seeds)

The equilibrium moisture content (EMC) is defined as the moisture content, at which the drying rate approaches zero. From Fig (2), this value could be obtained as (0.3 to 0.29) and (0.26 to 0.25) g H₂O / g DM, respectively for sun (solar) and oven dried leathers. The overall drying rate values (K-values) obtained from Henderson – Pabis equation have been found to be (0.135 to 0.1409) and (0.2109 to 0.2268) g H₂O / g DM. h for sun (solar) and oven dried mulberry sheets, respectively with drying rate for oven drying being 58.7% higher than those of sun (solar) drying.

3.5.3. Modelling drying curves of black mulberry sheets

Table (5) summarizes the statistical parameters of drying curves as analyzed by the aforementioned 3 models. As seen, page model failed to represent the drying curves of black mulberry sheets, since the average R² – value was low (0.8684) and the average value of SSE, RMSE and X² were 1890.9, 26.72 and 409.07, respectively. Also, average p% - value was 18.43% being higher than the maximum acceptable level of 15%. [38] On other side, both Henderson – pabis and Wang – Singh equation are suitable for prediction of moisture content during drying of Mulberry sheets. They recorded high R² – values (0.989 and 0.992. respectively). Also, SEE, RMSE and X²- values were much lower than those

of page – equation (1890.9 and 1536.45; 8.996 and 9.315) as well as (121.99 and 117.06), respectively for Henderson – pabis and Wang – Singh equation, respectively. The p% - values of both equations were lower than the limit of 15%, being in the average range of 7.37 and 11.15%, respectively. The results of statistical analysis proved that the moisture movement inside the sheet layer is mainly by gravity and water molecule diffusion, while the contribution of vapor (gas) diffusion is minor, in opposite to drying pattern of seeds and whole fruits, which contain a long phase of vapor (gases) diffusion and obey the Page model. The pattern of drying curves and drying rates of black mulberry leather obtained in the present work agree with those reported before. [19, 20, 39] They reported lower X² – and higher R² – values for Henderson and Pabis model in presenting drying process of mulberry fruits.

3.6. Quality attributes of dried black mulberry leather

Quality attributes include major chemical composition, sugar analysis and biochemical active components of the black mulberry leather subjected to different drying conditions as given in Table (6). As seen, the moisture content of fresh fruits of black mulberry was 83.48%, which is in the range reported before for black mulberry fruits grown in Egypt.

[30, 40, 41] The fresh fruit is rich in total carbohydrates (12.35%) and contain protein, lipids, ash and crude fibers in ratio of 1.26%, 0.638%, 0.82% and 1.27%, respectively. Drying led to reduce the moisture content of fruit puree to the level of 16.20% to 17.83%, which considered as safe for storage of fruit leather (less than 20%). [42, 43]

During drying, some biochemical reactions took place, which affected the final level of the different components in the produced leather. The content of crude protein was increased to levels lower than those expected for dried products. Protein level was in the range of 1.26 to 2.21% in the dried fruit leather. According to research reported before, [44-46] Protein losses during drying of fruit leathers could reach level of 33% due to protein denaturation, Release of amino acids from the proteins and browning reactions. Lipids level in the dried leather was also only 1.85 to 1.95 %, being lower than expected, may be due to either enzymatic hydrolysis and – or lipid oxidation because of the thermal treatment and drying temperature. [47] Crude fibers content reached Levels of 3.76% to 4.125% in the dried leather. Losses in fiber content was likely due to thermal degradation resulting in hydrolysis of fiber pre-courses such as pectin, cellulose and hemicellulose as well as due to forming a complex with proteins and phenols. The ash content of fresh black mulberry fruits was 0.82% and it was increased to the level of 3.76 to 5.25% in the dried leather. According to Koyuncu et. al. [48]. The black mulberry is rich in potassium followed by sodium and phosphors, which is a potential contribution to human nutrition. The total carbohydrates in fresh fruits are about 12.35% and this ratio was increased to the level of 69.80 to 71.18% after drying, so it could be considered as a good source of energy.

Table (6) includes the results of HPLC-analysis of the individual sugars in black mulberry fruits and their dried leathers. As seen, the major sugars present in fruits of black mulberry are glucose and fructose beside a small amount of sucrose and minor amounts of pentose sugar (ribose). Glucose and

fructose are present in equal amounts with a ratio (Glucose / fructose) of 1.03.

The total amount of reducing sugars is 10.67% (FW) and making about 90.8% of total sugars. Drying led to removal of moisture and concentration of present solids. Glucose concentration was increased by 252 to 261%, while that of fructose was slightly lower 233 to 249%, may be due to the formation of furfural in the dried product. The ratio (Glucose/Fructose) remained in the range of 104 to 110%. The ratio of sucrose was substantially increased from 1.03% in fresh fruits to be dominating in the dried leather (17.42 to 17.85%) due to the added sugar during the preparation of puree mix. However, the ratio of reducing sugar / total sugars remained in the range of 59.90 to 63.5%. The total sugar content of dried leather ranged between 44.26 to 44.57% making about 55% of the total solids present in the black mulberry leather, while the rest solids (45%) are those of proteins, fats, fiber, ash and minor components. The sugar components of fresh black mulberry given in the present work agree with those reported before [49-51]. Deseeding of black mulberry puree before drying led to significant differences in ribose content, while difference in values of glucose, fructose, sucrose and total sugars were minor between sun (solar) and oven drying or deseeding treatment of the puree before drying. The final total sugars of dried leather (44.26 to 44.33%) obtained in the presence work agree with those reported by Wang et. al. [1] The obtained results agree with those reported before [52, 53].

Table (6) includes also the active biochemical compounds found in Egyptian black mulberry fruits and how they have been affected by the drying method. As seen, the total phenolic compounds present in tested black mulberry fruits was found to be 323.33 mg/100g FW, which agree to a great extend with the values reported before [40, 41, 54, 55]. They reported total phenolics value for fresh black mulberry fruits, includes those grown in Egypt, between 234.6 to 485 mg/100g FW.

Table 5: Statistical parameters of drying models applied to black mulberry leather

Parameters	Henderson-Pabis equation				Page equation				Wang and Singh equation			
	Sun with seeds	Sun without seeds	Oven with seeds	Oven without seeds	Sun with seeds	Sun without seeds	Oven with seeds	Oven without seeds	Sun with seeds	Sun without seeds	Oven with seeds	Oven without seeds
R²	0.987	0.975	0.999	0.993	0.889	0.906	0.835	0.842	0.992	0.9863	0.9963	0.9928
RMSE	10.502	16.734	3.135	5.6131	23.575	27.798	26.490	29.268	9.237	13.235	6.266	8.528
X²	124.081	315.059	11.621	37.236	625.256	869.376	63.795	77.878	139.623	197.063	46.413	85.965
SSE	1985.304	5040.945	127.830	409.599	10004.10	13910.02	9122.66	11136.672	1535.853	3153.008	510.54	945.615
P %	6.40	15.74	2.24	5.10	15.88	10.19	24.14	23.54	9.90	15.19	8.50	11.03
K: g H₂O /g DM.h	0.135	0.1458	0.2109	0.2268	-	-	-	-	-	-	-	-

Table 6: Quality attributes of dried black mulberry leather (on fresh weight and dry weight)

Component	Fresh Black Mulberry	Sun (Solar) drying		Hot air drying	
		Not de-seeded	De-seeded	Not de seeded	De-seeded
Major Composition and nutritional value					
Moisture	83.48±1.20 ^A	17.83±0.09 ^B	17.10±0.06 ^B	16.90±0.06 ^B	16.20±0.06 ^B
Crude protein	1.26±0.33 ^B	2.16 ±0.12 ^A	1.91 ±0.08 ^A	2.21 ±0.12 ^A	1.39±0.09 ^B
Fat	0.638 ±0.39 ^B	1.85 ±0.06 ^A	1.807 ±0.14 ^A	1.93±0.03 ^A	1.72±0.06 ^A
Ash	0.82±0.02 ^D	4.46±0.06 ^C	5.25 ±0.12 ^A	5.19±0.07 ^A	4.92±0.03 ^B
Fibers	1.27±0.20 ^C	3.89±0.05 ^B	4.125 ±0.06 ^A	3.76±0.06 ^B	3.95±0.25 ^{AB}
Total carbohydrate	12.35±0.30 ^B	69.81±0.43 ^A	69.80 ±0.54 ^A	69.96±0.17 ^A	71.18±0.36 ^A
HPLC Sugar Fraction					
Ribose (g/100)	0.158±0.00 ^C	0.48±0.00 ^{AB}	0.36±0.00 ^D	0.51±0.00 ^C	0.44±0.03 ^B
Glucose (g/100)	5.33±0.12 ^B	13.56±0.00 ^A	13.52±0.00 ^A	13.44±0.01 ^A	13.48±0.03 ^A
Fructose (g/100)	5.18±0.03 ^C	12.32±0.00 ^B	12.10±0.05 ^B	12.79±0.11 ^A	12.94±0.03 ^A
Sucrose(g/100)	1.03±0.01 ^C	17.73±0.01 ^A	17.44±0.00 ^B	17.85±0.03 ^A	17.42±0.02 ^B
Total sugar%	11.69±0.34 ^B	44.33±0.36 ^A	44.26±1.06 ^A	44.57±0.66 ^A	44.28±0.50 ^A
Reducing sugar%	10.67±0.52 ^D	28.13±0.52 ^A	27.34±0.44 ^B	26.70±0.50 ^C	26.86±0.37 ^C
Biochemical Components					
Total antioxidant %	80.53±0.46 ^A	75.82±0.39 ^B	72.37±0.58 ^C	70.33±0.42 ^D	67.20±0.91 ^E
Total phenolic (mg/100 g)	323.33±1.20 ^A	293.33±5.84 ^B	290.67±6.69 ^B	201.67±3.71 ^C	200.33±6.33 ^C
Total flavonoids (mg/100 g)	281.93±9.58 ^A	239.33±3.76 ^B	230.67±0.33 ^B	198.67±1.45 ^C	180.67±3.84 ^D
Total anthocyanin (mg/100g)	222.6±2.39 ^A	198.53±0.22 ^B	189.53±0.43 ^C	177.57±1.02 ^D	170.20±2.92 ^E

Note: Means in the same row with different superscripts are significantly different ($P < 0.05$)

The fruits contain also considerable number of total flavonoids (281.93 mg/100g FW) and total anthocyanin pigments (222.60 mg/100FW). The antioxidative activity of the fresh fruits was 80.53%. Drying fruit pulp to produce mulberry fruit leather resulted in 9.3 to 38.04% reduction in total phenolics, 15.11 to 35.92% in total flavonoids and 10.81 to 23.54% in total anthocyanin pigments. The total antioxidative activity of the fresh fruit was reduced by 9.41 to 16.55% during drying. Sun drying resulted in higher values for retention of total phenolics, total flavonoids, total anthocyanins and higher antioxidative values compared with those of oven drying, may be due to the differences in temperature and air velocity (air stream) applied in both methods, which affect the oxidative degradation rate of the active components. Also, black mulberry leather sample containing seeds showed higher values in all tested active compounds compared with those of deseeded samples indicating the higher content of those compounds in black mulberry seeds. The obtained results agree with those researches reported before [52, 53]. They reported that phenolic compounds were highly susceptible to heat during drying process especially those ovens dried and in the presence of >1% pectin in mulberry leather due to increase in required drying time.

3.7. Scanning Electron Microscopy (SEM) of Black mulberry leather

Figure (4) shows the image for microstructure of cross-sectional areas of black mulberry leather samples at 800x magnification. As seen, image of leather obtained from deseeded black mulberry fruits (image 1 and 4) showed smooth homogenous structure with good intensity and compact light speckling. This may be due to the use of pectin (1.25%), which implies the restructuring of the used pulp through gelation leading to improving the texture of the obtained leather. The small spots (pores) appeared in the image (1 and 4) denotes the places where water was present in the formulation. Voids (dark zones) in the images presents surfaces of water diffusion outside during drying. Some roughness could be observed on the surface due to air flow on the surface (face) during drying process. Image of black mulberry leather prepared from non-deseeded pulp (images 2 and 4) show some crakes and more cohesion and disordered microstructure formed by gelation of pectin added in to the pulp and by water evaporation. Rougher surfaces seen in the images are due the incomplete homogenization of fibers in the matrix of the obtained leather. White spots in the images indicate the internal bonds between the components of the presence of seeds. No remarkable differences could be observed between sun (solar) and oven dried samples. The obtained results agree with those reported before [56, 57].

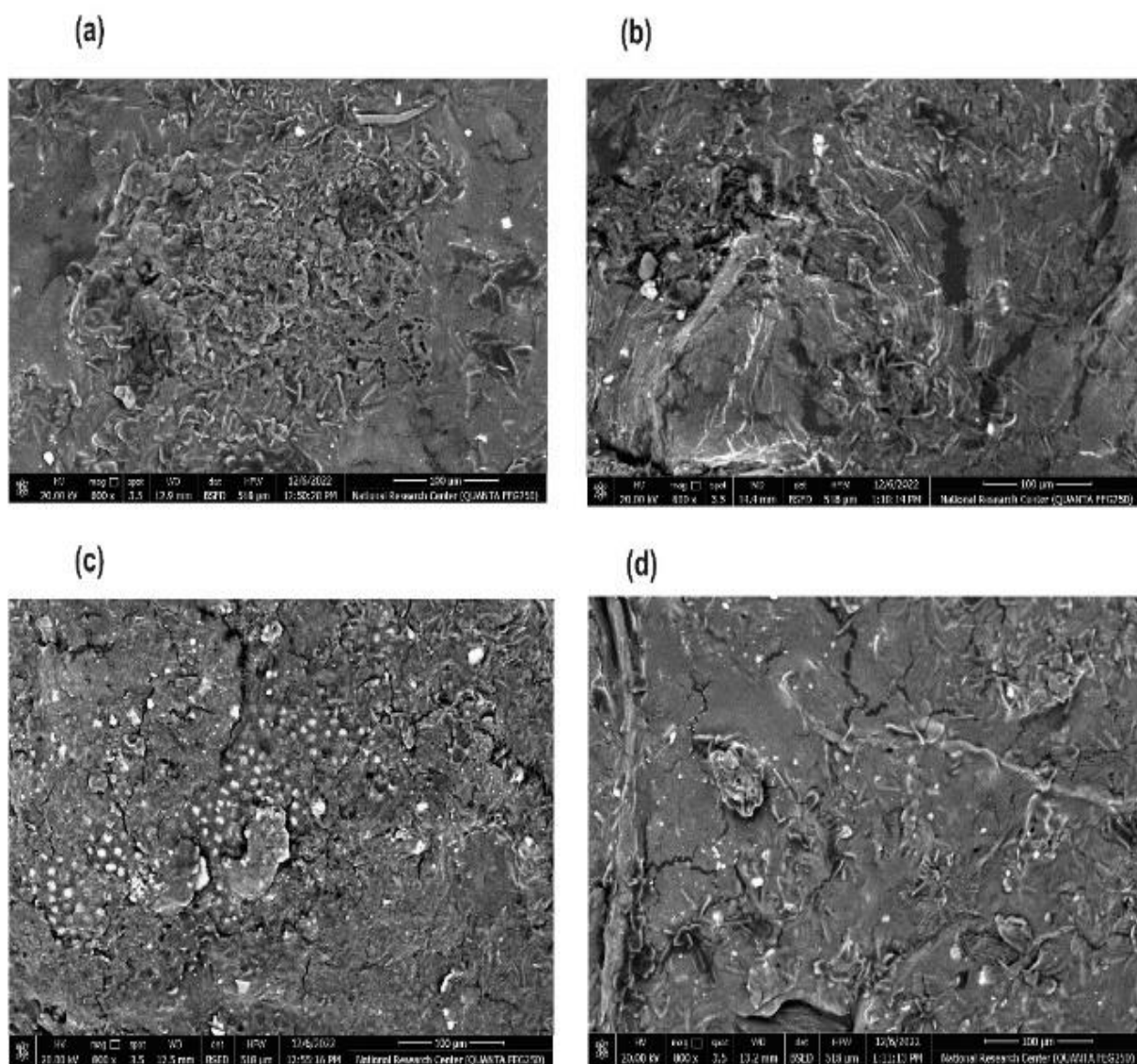


Figure 4: SEM: images of black mulberry fruit leather (a = Sun without seeds, b = Oven without seeds, c = sun with seeds and d = Oven with seeds)

3.8. Microbiological quality of black mulberry leather

Food safety is of great concern for the consumer, also for dried foods. Therefore, black mulberry leather samples were subjected to microbiological tests during 10 months of storage and the results are given in Table (7). As seen, all tested samples showed no growth of bacterial cells or yeasts and molds during the first 5 months of storage. This could be referred to the heat treatment of the pulp before drying from one side, and due to the low water activity (a_w) values of the obtained dried leather, from other side, which was lower than $a_w = 0.6$ (Table 3) indicating that these values are below the a_w -level for microbial growth. Furthermore, the presence of high amounts of phenolic compounds and flavonoids (Table 6) in the dried leather could contribute to the inhibition of microbial growth. Starting from the sixth month, microbial growth has

been observed in the tested mulberry leather samples and recording levels of 2.02 to 2.32 log CFU for bacterial count and 2.02 to 2.12 log CFU for yeasts and molds. The microbial load was slightly increased during the progress of storage period and reached levels of 2.28 to 2.62 log CFU for bacterial count and 2.21 to 2.29 log CFU for yeasts and molds. Oven dried mulberry leather samples showed slightly higher bacterial and yeast load than those sun (solar) dried, where leather samples containing seeds showed lower yeasts and mold load than those deseeded samples, may be due to the role of seeds content in assisting inhibition of microbial growth. The obtained results agree with those reported before [58], and comply with the safety regulation [59, 60], where the level of yeasts and molds should not exceed 100 CFU/gm in dried fruit leather.

Table (7): Log number of total bacterial count (TBC), yeasts and mold of Egyptian black mulberry leather during storage

Storage (month)	Total bacterial count				Yeasts and molds			
	Oven dried		Sun dried		Oven dried		Sun dried	
	Non- de-seeded	De-seeded	Non- de-seeded	De-seeded	Non- de-seeded	De-seeded	Non- de-seeded	De-seeded
0	0±0 ^{iA}	0±0 ^{iA}	0±0 ^{gB}	0±0 ^{gB}	0±0 ^{eA}	0±0 ^{eA}	0±0 ^{cA}	0±0 ^{cA}
2	0±0 ^{eA}	0±0 ^{eA}	0±0 ^{gA}	0±0 ^{fA}	0±0 ^{eA}	0±0 ^{eA}	0±0 ^{cA}	0±0 ^{eA}
4	0±0 ^{eA}	0±0 ^{eA}	0±0 ^{fA}	0±0 ^{dA}	0±0 ^{eA}	0±0 ^{eA}	0±0 ^{cA}	0±0 ^{eA}
6	2.31±0.01 ^{dA}	2.32±0.02 ^{iA}	2.02±0.02 ^{dC}	2.06±0.02 ^{cD}	2.02±0.02 ^{dD}	2.12±0.03 ^{dB}	0±0 ^{cE}	2.02±0.02 ^{dD}
8	2.55±0.01 ^{bA}	2.38±0.00 ^{gB}	2.02±0.02 ^{bE}	2.27±0.03 ^{aC}	2.19±0.01 ^{bD}	2.23±0.00 ^{bC}	2.02±0.02 ^{bE}	2.20±0.00 ^{bD}
10	2.62±0.01 ^{aA}	2.41±0.00 ^{fB}	2.28±0.02 ^{aC}	2.29±0.01 ^{aC}	2.27±0.01 ^{aC}	2.29±0.01 ^{aC}	2.21±0.02 ^{aD}	2.27±0.01 ^{aC}

Note: Means in the same column with different superscripts are significantly different ($P < 0.05$)

4. Conclusion

Production of leather from mulberry fruit is a promising process due to its popular and nutritional importance. In this research work, trials were conducted to incorporate some hydrocolloids (xanthan, CMC and pectin) in the fruit pulp to enhance the quality of produced leather. Results proved that pectin (1.25%) was the most suitable polymer for production of black mulberry leather. Addition of pectin improved the consistency of the obtained fruit pulp, texture and sensory characteristics of the dried leather. Furthermore, functional components (phenolics, flavonoids and anthocyanins) were more retained in the leather containing pectin. Also, surface smoothness and homogeneity were improved as a result of pectin gelation during processing and drying. No significant differences were found between oven and sun (solar) dried samples. It could be recommended to apply sun (solar) drying for production of black mulberry leather to save energy and to get product with acceptable high quality.

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The authors have no fund.

6. Author Contribution

Marwa Sheir: research idea, Literature review, experimental work, writing-Original draft, Reviewing and Editing. Abeer El-Baz: research idea, experimental work, writing-Original draft, Reviewing and Editing. Entsar Mohamed: research idea, Conceptualization, Literature review, experimental work, Data analysis; Writing-Original draft, Reviewing and Editing, Supervision.

7. Declarations of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

8. Data Availability

Data are available upon request.

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