EFFECT OF SOME PROCESSING METHODS ON THE PHYSIOCHEMICAL PROPERTIES OF BLACK MULBERRY

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Abstract. *Black mulberry fruits have a significant role in human nutrition ,due to its antioxidant activity .However owing to their high moisture content and sensitivity to storage, fresh black mulberries have a very short life time . Hence, they are usually processed immediately after harvesting ,which some time reduce their nutritional value . This study was carried out to check the effect of some processing methods (drying, concentration, freezing) in processing dried black mulberry, concentrated juice, leather sheets (Nana Shatw), and frozen juice on some physiochemical properties (Vitamin C, Total Phenol, Total Anthocyanin and Antioxidant capacity) as well as flavour properties (sugar contents ,organic acid contents) and sensory parameters. For this purpose, samples were subjected to quantitative and qualitative analyses (spectrophotometric, HPLC technique) in order to monitor the changes in those parameters.*

The results shows that the most processed methods that cause reduction in Vitamin C levels in black mulberries fruit were concentrating fruit juice and drying the fruit , due to their related with elevated temperature . Conversely these processes increase the level of total Phenol(2520 µg GAE/g and 2490 µg GAE/g respectively), Total Anthocyanin(824 µg C3GE and 784 µg GAE/g respectively),and the Antioxidant Capacity which depend on those parameters .Ragarding to the study of the effect of the processing methods on the flavor of black mulberries fruit which based on the ratio of sucrose to organic acids. the results show that all the processed methods reduce the level of sucrose while concentrating juice displayed elevated levels of Fructose (7.24 g/100g) and Glucose (8.94 g/100g), followed by dried black mulberries which also exhibited a highest level of total sugar content (17.23 g/100g), and higher concentration of organic acids Drying process cause increase in the level of the all studied organic acids, (oxalic, citric, tartaric, and malic acids). Sensory evaluation results also indicated consistency in most parameters, suggesting that consumers can enjoy these products. Further studies are recommended to optimization of food processing to protect the phenolic compounds in blackberry fruits to minimise the antioxidant loss and improve the final product quality.

Keywords: *Black mulberry, drying, Leather sheet, frozen juice and Antioxidant.*

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1. INTRODUCTION

The extraction of juice is the first step towards the production of fruit-based products such as marmalade, concentrated juice , soft drinks, fruit leather (pestil), alcoholic drinks, sauce, and jelly . Many factors affect on components of the black mulberry juice quality, such as Black mulberry (*Morus nigra* L.) is a kind of juicy delicious fruits with extraordinary colour and special acidic flavour, that plays a significant role in human nutrition as antioxidant, and anti-inflammatory activities , anti-diabetic, liver-protecting, eyesight-improving, slimming preparations, anti-cancer properties and anti-hyperlipidemia [21,32].This is due to their rich content of phenolic compounds, including phenolic acids, flavonols and anthocyanins as well as being a source of vitamins (B and C), minerals (iron and calcium) [8,9]. The distinction of black mulberry fruit compared with the white and red mulberry one is, its higher content of phenolic compounds, especially anthocyanin and its attractive color. Recently extract of black mulberry used to produce gel formulation as an antioxidant and sunscreen [4].Many species of black mulberry are extensively cultivated in various regions around the world including the Kurdistan region of Iraq.

However due to its perishable nature, as soft fruit, Black mulberry have short shelf life, moreover they are susceptible to fungi growth therefore, they are usually processed immediately after harvesting. The main directions of processing are the production of dried fruits, juices, pastes, jams, wine ,frozen desserts, kome, pestil, and molasses [2]. The most common method of mulberry processing is drying. Many dehydration techniques used to produced dried mulberries including insolation in natural conditions, Hot air drying, for preservation of the physicochemical properties of dried mulberries, vacuum drying [35] and as well as ,freeze drying ,hybrid drying [7] and the application of microwaves for drying [33].

Production of mulberry leather (pestil) is another method for increasing the shelf life of these fruits. Pestil are very pro popular fruit in kurdistan region of Iraq ,they are considered as alternative products to fruits and a source of various nutritional elements including dietary fibers, carbohydrates, minerals, vitamins and antioxidants moreover, it has a pleasant appearance, and does not require cold storage [27]. Mulberry leather is a concentration of mulberry juice and pulps with the addition of starch, sugars, and additives and dried in thin layer form in the sun or oven [33]. There are other production methods for mulberry leather which differ in their components like starch, wheat bran, whole grain flours as well as other ingredients such as honey, milk and nuts [17]. as juice processing ,juice concentration,powder production from the juice[31], and producing probiotic dairy using juice [14] . To extract black mulberry juice, many apparatus used, such as Roller mechanism, rotary spiral and hydraulic press, among them the filter-press is the reliabile method for this purpose [16]. To freeze fruit juice, the juice pour into a container, leaving some space at the top to allow for expansion of the juice during freezes ,then the container tightly sealed and place it in the freezer.. In spite of the importance of food Processing in making tastier and more shelf-stable food, it can also maybe affecting the nutritional quality of foods for example, high temperature during some processing

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methods, results in losses of some vitamins and minerals.

This study aimed to evaluate the effect of some food processing methods(dried black mulberry by drying, concentrated juice and leather sheets by concentration, and frozen juice by freezing)which were most popular in kurdistan region of Iraq on some physiochemical properties (vitamin C, Total Phenol, Total Anthocyanin and Antioxidant capacity) and flavour properties (sugar contents ,organic acid contents) as well as sensory parameters.

2. MATERIALS AND METHODS

This study was conducted at the University of Sulaimany, College of Agriculture Science, Department of Food Science and Quality Control, between june to september 2023. fresh local black mulberry varieties were utilized for various food processing methods, including dried fruits, concentrated juice, leather sheets, and frozen juice. the quality of the products were assessed using certain chemical properties(sugar content, organic acids) as well as sensory evaluations.

2.1. Black Mulberry Processing

Initially, fresh black mulberry fruits (Figure 1a) are washing with water and arranging them on a hot air dehydrator tray on 55°C, for 15-24 hours to prepare the dried mulberry (Figure 1b). For preparing Frozen Juice (Freezo) (Figure 1d) an electric blender was used to extract the juice, then the extracted juice was filtered using a muslin cloth and poured into a glass jar without any additional ingredients, then transferred to polyethylene bags and kept on - 18°C till analysis. The extracted juice is used to prepare a concentrated Juice of Black Mulberry (Figure 1e) by placing the juice into a stainless steel pot and heated at 95°C for 5 hours, with continuous stirring until reaches a concentration of 60 brix, then poured into a sterilized, airtight glass jar , then the product cooled and stored at temperatures ranging from 4 to 20°C until analysis. Regarding to Black Mulberry Leather Sheet (Nana shatw) (Figure 1f), the extracted juice was heated at 95°C for 5 hours, and stirred continuously until it reached a concentration of 60 brix, Afterward, the concentrated mixture was spread on a grassed baking sheet and dehydrated in a food dehydrator at 50°C for 48 hours. Once dried to a non-sticky, flexible consistency, the black mulberry fruit leather sheets were sealed and stored for sensory evaluation .

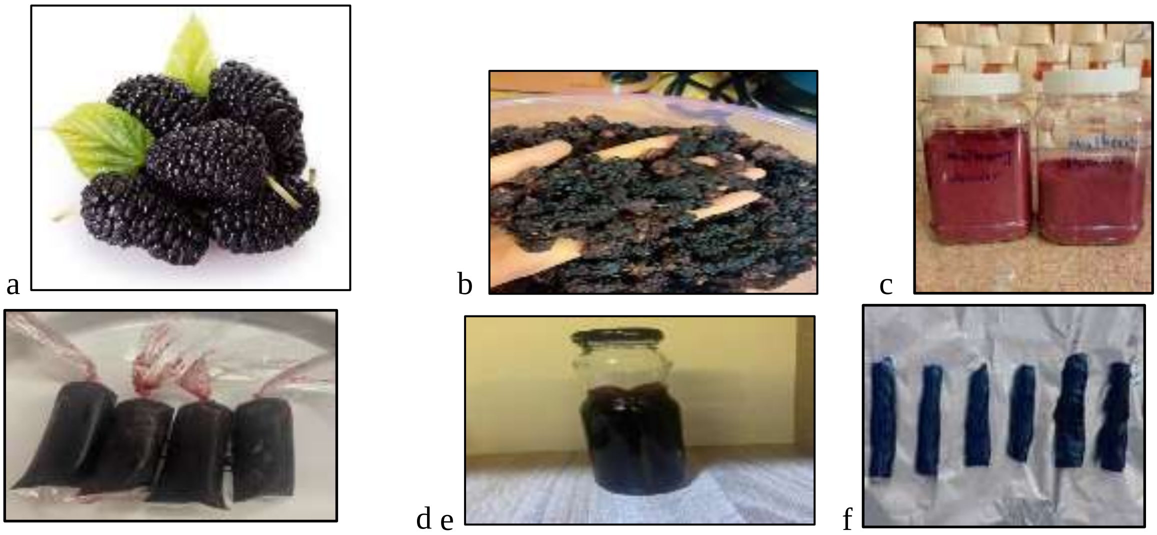

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Fig. 1.Shows picture of Processed products from fresh black mulberry (a. fresh black mulberry, b. dried mulberry c.mulberry powder ,d.frozen black mulberry ,e.concentrated juice of mulberry and f. Mulberry leather sheet).

2.2. Physico-Chemical Analysis of Black Mulberry Product

2.2.1. pH and Vitamin C content

The sample's effective acidity was directly determined using a pH meter (Hanna-HI 98103), [11]. Vitamin C content in the samples was analyzed using a modified HPLC method, [13]. which involve separation on a Fast Liquid Chromatographic (FLC) column (STR ODS-II, 1.3µm particle size, 50 x 4.6 mm I.D) with a mobile phase of (0.1 M) potassium phosphate pH (2.6), and (5 mM) octane sulphonate (ion-pair), and acetonitrile (9:1, A:B, V/V). Detection was performed at (270 nm) with a flow rate of (1.5 ml/min), and a temperature of (40°C). Eluted material sequences for standard compounds (25 µg/ml each) were determined. Sample preparation involved dissolving (10 g) in HPLC methanol: water (60:40 v/v), concentrating to (0.5 ml) with liquid N_2, adding mobile phase to reach (1 ml), and injecting (20 µl) on the HPLC column. Quantitative determination of compound concentrations was based on comparing peak areas of samples with the standards. The separation occurred on a Shimadzu 10AV-LC with UV-Vis 10 A-SPD spectrophotometer monitoring eluted peaks.

2.2.2. Analysis of Total Phenol (µg GAE/g)

To extract total phenolic compounds from fruits, (10 g) of fruit material underwent treatment with 80% ethanol (40 mL) at 80°C for 20 minutes in an inert atmosphere. The filtered extract was repeated and combined, then diluted to 100 mL with 80% ethanol. The resulting extract was quantified using the Folin–Ciocalteu method. A (1 mL) extract, mixed with (20 mL) deionized water, (1 mL) Folin–Ciocalteu's reagent, and (5 mL 20% Na_2CO_3), was measured for absorbance at (765 nm) after (30 minutes). Results were expressed as (µg gallic acid equivalent (GAE)/g of fw).

For determining total phenolic content (TPC) in black mulberry extract, a stock solution (4000 µg/ml) of Gallic acid monohydrate was prepared by dissolving 1000 mg in 250 ml of methanol). Standard calibration curves were created, ranging from (500 to 2500 µg/ml), by mixing these solutions with Folin-Ciocalteau phenol reagent and sodium carbonate, incubating, and measuring absorbance at (765 nm) in a Shimadzu 160A,UV-VIS Spectrophotometer . The resulting calibration curve was then used to assess the total phenol concentration in mulberry samples based on their absorbance [13], [18], [19] , [28].

2.2.3. Analysis of Total Anthocyanin (µg C3GE/g)

For total anthocyanin determination, a (1000 ppm) stock solution of cyanidin-3-glucoside standard (1 mg/ml) is prepared with ultra-pure water containing (0.1% HCl). Standard curves are plotted using solutions at different concentrations derived from the stock solution. Fruit anthocyanin content is extracted from (2 g) of fruits with (0.1% HCl (2 mL) in 96% ethanol), and (2% HCl (40 mL)). After centrifugation at (5500 rpm) for (10 min), the measurement mixture consists of the extract (10 mL) and (15 %) sodium bisulphite (4 mL). Following a (15-minute) reaction at room temperature, absorbance is measured at (520 nm) using a (UV 160A) spectrophotometer (Shimadzu, Kyoto, Japan), employing the molar absorbance value for cyanidin-3-glucoside (C3GE) at different concentrations (25-800 µg/ml). Results are

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expressed as (μg of cyanidin-3-glucoside equivalents/g of fw) [18], [19], [28].

2.2.4. Analysis of Antioxidant capacity DPPH (%)

The determination of DPPH radical scavenging activity followed the protocol outlined by [23]. A freshly prepared DPPH solution in methanol, consisting of 1 ml of 10-4 M DPPH, was transferred to a glass tube lined with aluminum foil. Subsequently, 3 ml samples of antioxidant solutions in methanol with concentrations of 0, 3, 1.25, 6.25, 12.5, 25, 50, 100, 200, and 400 μg ml-1 were added to the DPPH solution. Control tubes received 3 ml of pure methanol instead of the antioxidant solution. The mixtures were then incubated in the dark at room temperature for 30 minutes, followed by absorbance measurement at 517 nm against methanol. Ascorbic acid and Trolox served as standards. The percentage of DPPH scavenging activity was calculated using the formula:

$$DPPH\% = \frac{Ac - As}{Ac} \times 100$$

Here, Ac represents the absorbance of the negative control (containing the extraction solvent instead of the sample), and As is the absorbance of the samples. The results were expressed as EC50 (μg ml-1) [5], [8], [9].

2.2.5. Analysis of Sugar Content (g\100 gm)

Carbohydrate levels in black mulberry were determined using Fast Liquid Chromatography (FLC) with a sixpack A1 column (3 μm particle size, 50 x 4.6 mm I.D). The mobile phase, comprising (15 mM NaOH) spiked with (1 mM) barium acetate, was detected using a refractive index detector. Operating at a flow rate of (1.5 ml/min), 40°C temperature, and an injection volume of (20 μl), the standard elution involved glucose, sucrose, and fructose, each sequenced with their retention times. Samples were prepared by dissolving (1 gm) in (5 ml) deionized water, followed by centrifugation, filtration, and concentration analysis (ug/ml). This method, adapted from Cataldi et al. (Anal. Chem. 2000, 72, 3902-3907), employed a Shimadzu 10AV-LC with a Shimadzu RID-6A refractive index spectrophotometer [19].

2.2.6. Analysis of Organic Acid Content (g\100 gm)

The total oxalic acid , citric acid, tartaic acid, and malice acid in black mulberry product samples were determined by HPLC, and the methods were applied by [1].

2.3. Sensory Evaluation Of Black Mulberry product

For the sensory assessment of different black mulberry products, a 9-point hedonic rating scale was employed for each test. The points ranged from 1, indicating extreme dislike, to 9, representing extreme liking. A total of 12 panels were selected to evaluate the product, focusing on sensory properties such as color,odor, taste, sour taste, sweet taste, and flavor [17 , 27].

2.4. Statistical Analysis

The data, obtained from these experiments, comprises the means derived from three replications. The data underwent analysis through one-way ANOVA. Consequently, the XLSTAT program for the Windows (Version 7.5.2.) was utilized for further analysis. Following this, Duncan's test was employed to evaluate differences between samples, with a confidence interval of 95.00 %.

3. RESULTS AND DISCUSSION

3.1. Chemical Content in Black Mulberry Fruit Product

While some studies revealed that processing can alter and often decrease the levels of fruit antioxidants compared with the fresh ones , in contrast, other studies have shown that the antioxidant levels of processed fruits derived

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from e.g. sour cherry are maintained by the high recovery after processing [30]. **Table 1** presents the pH, Vitamin C, Total Phenol, Total Anthocyanin, and Antioxidant Capacity levels in the black mulberry product. There are no significant differences between the pH values of processing methods compared to the pH values of the fresh fruit. This observation aligns with a previous study conducted on black mulberry fruit and its products [19]. Hence, this study indicated that the processing methods did not impact the pH of the black mulberry fruit.

Table 1. Illustrated the chemical analysis for various black mulberry products.

Samples	Chemical analysis				
	pH	Vitamin C g\100gm	Total Phenol µg GAE \g	Total Anthocyanin µg C3GE\g	Antioxidant capacity DPPH %
Fresh fruit	4.160[a]	24.120[a]	1890[c]	700[b]	20.73[b]
Concentrated juice	3.880[a]	16.370[c]	2520[a]	824[a]	23.17[a]
Dried fruit	3.980[a]	20.750[b]	2490[b]	684[c]	20.95[b]
Leather sheet	3.640[a]	17.900[c]	1790[e]	654[d]	17.07[c]
Frozen juice	4.240[a]	24.980[a]	1820[d]	677[b]	19.51[b]

Different letters in a column indicate that the variations are statistically significant at the *p*<0.05 level, while the same letter indicates that the variations are not statistically significant.

Regarding to the effect of the processing methods on vitamin C (VC) content in black mulberry products, the results (table 1)shows that juice processing (frozen juice) maintain the level of vitamin C compared to fresh mulberry, while VC reduced in other processing methods ,since concentrated juice recorded a lower VC content. These findings align with the work of [20] on mulberry fruit. This is because Vitamin C is characterized by low thermal stability and a tendency to oxidize easily; therefore, each food process using elevated temperature causes loss of this vitamin compared to fresh fruit [15]. These losses can ranged between 20% - 80% depending on the temperature level, contact with oxygen and the duration of the processing technique.

In comparison of the effect of the processing methods on Total Phenol (TP) µg GAE/g values in black mulberry fruit . Concentrate juice of black mulberry demonstrated the highest TP content (2520 µg GAE/g), followed by dried mulberry ,whereas leather sheet decrease the level of TP ,as lowest TP value (1790 µg GAE/g) recorded after exhibit the fruit in this processing method . Frozen juice maintain 93% of the TP ,this result close to that of [36] who mentioned that TP content in the mulberry juice showed a slight decrease compared to fresh one , Similar results were obtained in the mulberry juice during pasteurization processes [34] this is due mashing and pressing of the black mulberry fruit led to the recovery of fruit polyphenols into the juice fraction. Frozen juice is preferred due some phenolics in fresh juice could have been linked to other compounds such as proteins, and results in producing large particles result in sediment formation [22].

The most important component of black mulberry is Anthocyanins, it have elevated levels that positively impact human health by preventing obesity, cardiovascular diseases, exhibiting antibacterial, anticancer, and anti-inflammatory properties [9]. The highest Total Anthocyanin (TA) content recorded in concentrated juice(825 µg C3GE/g) while drying cause decrease of TA (684 µg C3GE/g), these findings are close to the study of [6],who reported that drying decrease the anthocyanin levels in black mulberry fruits because of the degradation that increase with time and temperature of drying .Frozen juice has less effect on anthocyanin level ,the frozen product maintain its colour after freezing.[29] also found that 60–70% of the fruit anthocyanins were retained in the final juice, relative to the fresh fruit,this is due to that anthocyanin is water-soluble pigments,and the moisture levels not reduce during freezing.

The antioxidant activity of plants is mainly contributed by the active compounds present in them . Black mulberry fruit is acknowledged for its rich natural antioxidant content including TP and vitamin C. various methods exist to measure antioxidants . In this experiment, DPPH method for this purpose was used due to its simplicity and rapidity. No significant differences recorded between the fresh fruit and the frozen juice of black mulberry in antioxidant content . Notably, the highest level of antioxidant content (23.13 DPPH %) was obtained from concentrate juice followed by dried fruit (20.95) .Similar findings were obtained by [6] who reported that an increase in the temperature cause increase the antioxidant activity of the final samples. This is due to the increase in the total phenolic content cause an increment in the antioxidant activity of the sample due to a positive correlation between the TP and antioxidant activity .

3.2. Sugar Content in Black Mulberry Fruit Product

Organic acid to sugar ratio (the ratio of glucose to fructose) is a key criterion in characterizing in black mulberry fruit flavor. [25] also expressed that the balance between organic acids and sugars was important for desirable flavour of fruits in place of both consumer preferences and industry requirements Regarding to the sugar as shown in **Table 2,** all the processing methods used in this study cause decrease in the level of sucrose content compared to that of fresh black mulberry fruit (1.82 g/100g).Since sucrose hydrolyzes to fructose and glucose , the highest percent of these two monosaccharide (7.42 and 8.94 g/100g, respectively) obtained in Concentrated juice of black mulberry fruit.

while drying process cause increase in the level of total sugar since higher total sugar content was noted in dried mulberries (17.23 g/100g). whereas the frozen juice recorded lower amounts of fructose, glucose, and total sugar (5.11, 7.31, and 13.317 g/100g).

Table 2. Present the sugar content for various black mulberry products.

Samples	Sugar Content g/100gm			
	Sucrose	Fructose	Glucose	Total Sugar
Fresh	1.820^{a}	5.585^{ab}	7.360^{a}	15.030^{bc}
Concentrated juice	0.830^{b}	7.420^{a}	8.940^{a}	17.200^{a}
Dried	0.495^{b}	6.860^{ab}	8.88^{a}	17.230^{a}
Leather sheet	0.630^{b}	7.33^{a}	7.700^{a}	15.660^{ab}
Frozen juice	0.932^{b}	5.110^{b}	7.310^{a}	13.317^{c}

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Different letters in a column indicate that the variations are statistically significant at the *p*<0.05 level, while the same letter indicates that the variations are not statistically significant.

Sugar content plays a crucial role in the flavor profile and nutritional properties of fruits, These findings align with the study of [26] who reported a sugar level in black mulberries close to the current study.

3.3. Organic Acid Content in Black Mulberry Fruit Product

The essential components of the mulberries are the organic acids The production and composition of organic acids, the essential components of the mulberries , significantly influence its organoleptic characteristics. In the case of black mulberry fruit, prominent organic acids include oxalic, citric, tartaric, and malic acids [26]. The ratio between sugar and acid in the fruit exhibits a negative correlation, meaning that as the sugar content increases, the fruit tends to have a sweeter taste, whereas a decrease in sugar content results in a more sour taste [24]. The effect of processing methods used in this study on the content of organic acids in black mulberry fruit products is outlined in **Table 3**.

Table 3. Displaying the organic acid content for various black mulberry products.

Samples	Organic Acid g/100gm			
	Oxalic Acid	Citric Acid	Tartaric Acid	Malic Acid
fresh	0.996[a]	2.380[b]	0.689[c]	6.330[b]
Concentrated juice	1.824[a]	2.600[b]	0.970[b]	10.070[a]
Dried	1.880[a]	5.870[a]	1.070[a]	10.187[a]
Leather sheet	1.824[a]	3.110[b]	0.915[b]	9.942[a]
Frozen juice	0.959[a]	1.930[b]	0.600[d]	5.300[b]

Different letters in a column indicate that the variations are statistically significant at the *p*<0.05 level, while the same letter indicates that the variations are not statistically significant.

Drying process cause increase in the level of the all studied organic acids, (oxalic, citric, tartaric, and malic acids),since highest level of these acids were observed in dried black mulberries (1.88, 5.87, 1.07, and 10.187 g/100 g). Followed by concentrated juice except the citric acid. .Conversely, frozen juice exhibited the lowest organic acid content (0.959, 1.93, 0.6, and 5.3 g/100 g) . The presence of organic acids in black mulberry fruits plays an important role in promoting overall health owing to the alkaline property , it could also influence food decay [12].

3.1. Sensory Evalution in Black Mulberry Fruit Product

The outcomes of the sensory evaluation are presented in **Table 4.** The panelists assigned scores include some parameters(color, odor, Sour taste, and sweet taste to each product, revealing statistically insignificant differences. In general, these parameters exhibit stability throughout food processing. However, there are notable differences among the effect of various processing methods on odor, taste, and flavor on the black mulberry products, since,The highest scores for these parameters were recorded in fresh black mulberries (8.333, 8.5, and 8.25) respectivly , whereas the lowest scores were recorded for dried black mulberries (6.667, 6.75, and 6.5) respectively.

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Table 4. The sensory evaluation of various black mulberry products.

Samples	Sensory Evaluation					
	Color	Odor	Over all Taste	Sour Taste	Sweet Taste	Flavor
Fresh	8.333[a]	8.333[a]	8.500[a]	7.917[a]	6.833[a]	8.250[a]
Concentrated juice	8.417[a]	7.667[ab]	8.000[ab]	8.083[a]	7.500[a]	8.083[a]
Dried	8.167[a]	6.667[b]	6.750[b]	6.667[a]	6.500[a]	6.500[b]
Leather sheet	7.917[a]	6.750[b]	7.583[ab]	7.417[a]	7.250[a]	7.500[ab]
Frozen juice	8.500[a]	7.083[ab]	7.417[ab]	7.417[a]	6.833[a]	7.333[ab]

Distinct letters within a column signify statistical significance at the *p*<0.05 level, indicating variations between groups. Conversely, identical letters denote a lack of statistical significance, suggesting no significant differences between the compared

Dried mulberries can be stored in a sealed bag or container at room temperature away from heat and direct sunlight or in the refrigerator for up to one year to maintain their freshness, Additionally, the option to store them in the form of leather sheets or frozen juice and concentrated juice allows for extended preservation [27]. These measures contribute to providing a longer shelf life for black mulberry fruit used in food processing [33].

4. CONCLUSIONS

The results revealed distinctions among the effect of processing methods used in this study on some physicochemical properties of black mulberry,while elevated temperature related to some food processing (concentrated juice ,dried and leather sheet) resulted in decrease of vitamin c ,they increase of total polyphenols, and concequently antioxidant capacity of black mulberry products. Frozen juice was the best processing method to maintain vitamin c and Anthocyanin. Optimisation of food processing could help to protect the phenolic compounds in fruits which might be helpful for the food industry to minimise the antioxidant loss and improve the final product quality.

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