Minufiya J. Agric. Res. Vol. 40 No. 1: 41 - 56 (2015) **"http://www.mujar.net"**

CHEMICAL AND TECHNOLOGICAL STUDIES ON BLACK MULBERRY JUICE AND ITS CONCENTRATE

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(Received: Dec. 8, 2014)

ABSTRACT: Fresh Black mulberry was mechanically extracted and clarified by using gelatin and (UF) ultrafiltration process. The clarified juice was concentrated using rotary evaporator and freezing process.. Physico-chemical properties as well as chemical constituents and microbiological aspects was determined, The juice yield of 62.80 % with a moisture content was 81.20 %,. Total soluble solids (TSS%), pH value and acidity were16.4%, 5.49 and 0.341% respectively. Color index at (420nm) was 1.259. Clarity at (660 nm) was 35.9%. The major components were total sugars which recorded 12.80 %. Reducing sugars represents 11.20%. The juice contained 28.22 mg/100g fresh weight of L-ascorbic acid. Total antioxidant capacity (%), total flavonoids, total anthocyanin (mg/100g) and total phenols, were 53.72 %, 52.32 (mg/100g), 212.77 (mg/100g) and 434.61 (mg GAE /100g) respectively. Fourteen phenolic compounds were detected by HPLC. The obtained results during and after storage at room temperature 25C º ± 5C º for four months, declared that the clarified mulberry juice previously clarified gelatin ,having a lower stability than that clarified by Ultrafiltration process. Generally the juice was microbiologically safe. The sensory properties of mulberry nectar indicated that the prepared nectar from its concentrate after the end of storage is accepted and cope with the previous obtained data.

Key word: *Black mulberry juice, clarification, reconstitution, gelatin agent, ultrafiltration process.*

INTRODUCTION

The consumers interest for healthier and natural products has been growing and contributing to the consumption of lighter and refreshing products, such as fruit juices and bases fruit drinks (nectar, cocktails and drinks) (Matta *et al*., 2004). From the Numerous literatures dealing to mulberry fruits, the various cultivars of mulberry fruits have nutritional and therapeutically properties, as they contain the phytochemical compound particularly the natural antioxidant agents. In the future, we should care for accelerating planting mulberry trees in places, which can not affect the agricultural area.

In Egypt, approximately 3 million old mulberry trees in the Delta area along the canals and roadsides were counted (Megalla, *et al* 1997). Mulberry, a member of the family *Moraceae*, is a deciduous tree distributed in the warm, moderate, and subtropical regions of Asia, Africa, North America, and Southern Europe. There are more than 20 species of mulberry reported so far, and five of the species *Morus alba, M. nigra, M. rubra, M. indica,* and *M. laevigata* have edible fruit (Awasthi *et al*., 2004).

Black mulberry fruits are consumed as fresh fruit or in the form of various confectionary products such as jam, marmalade, frozen desserts, pulp, juice, paste, ice cream, and wine. It is expected that the consumption of black mulberry fruit, with its delicious slightly acid flavor, extraordinary taste, and medicinal use will increase in the near future Koyuncu (2004). Black mulberry is a fruit known not only for its nutritional qualities and its flavor, but also for its traditional use in natural medicine, as it has a high content of active therapeutic compounds. (*Morus. nigra*) fruit is a good source of several phytonutrients and contains high amounts of total phenolics, total flavonoids, and ascorbic acid. Also, the

fruit has a pleasant taste with a slightly acidic flavor and an attractive dark red color Ozgen *et al* (2009).

Amin and Attia (2003) reported that the weight of black mulberry fruit was 1.89 g, fruit dimensions (length 1.93 cm and diameter 1.23 cm), size 1.66 cm^3/fruit, juice yield was 76.54 %, pomace yield 23.46%, total soluble solids (T.S.S) 17.70% and the pH value was 5.70. Black mulberry has a moisture content of 79.68%, crude protein 9.50%, ash 5.22%, titratable acidity (as malice acid) 0.34%, reducing sugars13.48%, non-reducing sugars 0.22%, total sugars13.7, crude fiber 5.36%, nitrogen free extract 73.87% d.w. They also added that the black mulberry fruits are rich in anthocyanin (200.96 mg/100gm) on fresh weight basis.

Darias-Martín *et al* (2003) stated that the physical parameters of black mulberry were as follow, volume mass 1.06-1.007 g/cm, pH value was 3.10-3.36 and the TSS ranged between 13.00 to 17.50 %. They also found that the black mulberry has 3.12- 4.27 g/L of tannins, while the anthocyanin content was 2000 to 2600 mg/L.

Clarification process is very important technique for several fruit juices, to improve their qualities such as overall acceptability for the consumers and overcome the disadvantage of hazing and turbidity phenomena of some fruit juices especially during storage. (Stocke. 1998).

Ultrafiltration process (UF) has been used successfully in the clarification of pressed or refiltered of apple juice and other pressed fruit juices (Kirk *et al*., 1963). Clarified juice coming from the Ultrafiltration process can be commercialized or submitted to a concentration process in order to obtain a product suitable for the preparation of juice and beverages, (Cassano *et al*., 2003).

The concentration of fruit juices requires the part removal of water without changes in the composition of solids, leaving all the original solid components, such as fruit sugars, minerals, and vitamins, to the more concentrated solution. Concentration of fruit juices, a major unit operation in the fruit-processing industry, is of critical importance as it determines the quality of the final product such as flavor, color, aroma, appearance and mouth feel. Color kinetics is the most important aspect of the successful processing of mulberry fruit, color is usually the first property the consumer observes. Fazaeli *et al* (2011).

Because of the black mulberry juice is considered an important a new and untraditional juice, which has a pleasant taste and aroma, but it has more of hazing precursors, so some of attempts of this work were conducted to reduce this phenomena, followed by the concentration process. The effect of storage at room temperature on the properties of reconstituted mulberry juice, as well as the sensory properties of mulberry nectar, were determined.

The aim of the current research was to study:

The physico-chemical constituents of black mulberry fruits. The optimum concentration of its effect on some properties of clarified juice. The influence of Ultrafiltration process and also gelatin agent on some estimated parameters of clarified juices. The effect of various techniques of concentration processes using (Rotary evaporator and basket centrifuge equipment's') on physico-chemicals constituents and sensory properties of reconstituted juices. The influence of storage conditions on physico-chemical constituents and microbial evaluation on reconstituted prickly pear juices as well as mulberry juice. The evaluation of sensory properties of the nectar prepared from concentrated prickly pear juices and mulberry juice after the end of storage period.

MATERIALS AND METHODS

Materials:-

Black mulberry fruits (*Morus nigra* L.) was obtained from Giza district Giza Governorate "Egypt". The all fruits were at

ripe stage and collected in the year of 2012/2013.

Other materials used:

White sucrose sugar was purchased from the local market. Citric acid, sodium benzoate, potassium sorbate, Gelatin and celite were obtained from El- Gamhouria Trading Chemicals and Drugs Company, Egypt. The microbiological media (total bacterial count (TBC) using nutrient agar medium and yeast & mold count using potato dextrose agar (PDA), were obtained from El- Gamhouria Trading Chemicals and Drugs Company, Egypt.

Methods:

Technological methods

Extraction of black mulberry juice:

The ripe Black mulberry fruits were carefully sorted, graded, then proper washing, and extracted by pulper machine, which applied at (Food Technology Research Institute, Agriculture Research Center). The resultant pulp was homogenized, sieved with pressing well. The filtered juice was subjected to further processing.

Clarification process by gelatin agent:

Gelatin agent (0.15 g/L) was added to the homogenized juice (5000 rpm. for 5 min.). Sodium benzoate at the concentration of 0.025 % and also 0.025 % of potassium sorbate were added as preservative agents, and then left for 12 hours in a refrigerator. Celite as filter aid was used to facilitate the filtration operation by filter paper, using Buchner funnel. The clear juice was pasteurized at 95ºC for 5 min. in a water bath and directly cooled with running tap water to the ambient temperature.

Ultra- filtration: (UF) Clarification:

Ultra-filtration was performed by using a laboratory pilot plant supplied by Tech-sep (Richelieu, (Québec) Canada) equipped with a Koch tubular membrane carboser modules (type Series-Cor HFM-7 , PVDF, nominal molecular weight cut-off 50 (10^3 Dalton), surface membrane area 0.16 m^2, No. tubes 7, UF membranes M8, membrane diameter 6 mm, bursting pressure 40 bar, service pressure 15 bar, service pH 0-14). Experiments were carried out in the batch concentration mode to concentrate the juice up to a recovery factor of 42%. The membrane module was rinsed with tap water for 30 min after the treatment of the juice; then it was submitted to a cleaning procedure with the alkaline detergent Ultrasil 10 (El- Gamhouria Trading Chemicals and Drugs Company, Egypt) at a concentration of 0.2 % w/w % and at a temperature of 40 ºC for 60 min. A final rinse of the system with tap water for at least 20 min was carried out. Body device was manufactured in the Synergy Egypt Company (Mohandeseen, Giza, Egypt) .Sodium benzoate at the concentration of 0.025 % and also 0.025 % of potassium sorbate were added as preservative agents, then the clear juice was pasteurized at 95ºC for 5 min. in a water bath and directly cooled with running tap water to the ambient temperature.

Vacuum concentration:

Clarified mulberry juice was concentrated by rotary evaporator apparatus using an oil vacuum pump at 28 Hg and a temperature not exceeding 45ºC. The process was continued until the concentration process reached 35 Brix of total soluble solids. Then the concentrated clear juice was packed into 250 ml. sterilized brown bottles, pasteurized at 90ºC for1min. in water bath , and cooled directly using tap water. The juices were subjected to the proper analysis.

Freezing concentration method: (Basket centrifuge):

Clarified mulberry juice were put inside the refrigerator cubes and frozen at -18ºC for 24 hours. The frozen juice was placed in Basket centrifuge at 7000 rpm under atmospheric conditions to separate the concentrated juice from the formed ice, which remains inside the basket. This operation was repeated 3 times to obtain a concentrated juice of about 25 Brix.

The concentrated clear juice was packed into 250 ml. sterilized brown bottles,

pasteurized at 90 ºC for 1 min. in water bath, and cooled directly using tap water.

All porously previously juices were stored at ambient temperature (25C+5C) for four months and subjected to the proper analysis.

Reconstitution process

To analyze and evaluate the physico-chemical constituents as well as the sensory properties of the concentrates, the concentrates were reconstituted into the initial total soluble solids (16.4) as found in the fresh juice.

Preparation of black mulberry nectars:

To prepare black mulberry nectar, 40% from the single strength clarified reconstituted juice was used as participation ratio. 0.3% of citric acid was added to the formula. The final formula was adjusted to 16 Brix by adding sucrose solution. The nectar was packed into 200 ml. glass bottles and subjected to the pasteurization process at 90 C for 1 minute, directly cooled with running tap water and subjected to organoleptic evaluation.

Analytical methods:
Chemical constituents

Moisture, total soluble solids (T.S.S.) as Brix, total acidity, pH value, L ascorbic acid, crude fibers, ash and protein contents were determined according to A.O.A.C. (2012). Total sugars and reducing sugars were determined according to the method described by Somogy (1952) and Nelson (1974). Non-reducing sugars was calculated by difference

Physico-chemicals method

Viscosity was measured using Brookfield viscometer Model DV-III Rhemeter, at 25ºC using Spindle No. (21) LV and rotating speed of 50 rpm for one min. in both single strength turbid juice and in the case of clarified juice.

Browning index was measured according to the method reported by A.O.A.C. (2012), using (Shimadzu Spectrophotometer UV-120-02) at wave length of 420 nm. Color values were determined at (Food science departs. Faculty of Agric., Cairo Univ., Giza, Egypt), using Hunter Lab model D-25 color and color difference meter according to the method of Cano *et al* (1995).

Pectin content was determined as described by Lees (1975).

Clarity was measured at 660 nm according to the methods of Krop and Pilnik (1974).

Antioxidant method

The total carotenoids were determined according to the method described by Askar and Treptow (1993). The antioxidant activities of the aqueous juice extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical following the method described by Braca *et al.,* (2001). Total anthocyanin content of black mulberry juice was measured according the method described by Cevallos-casals and Cisneros-Zevallos, 2004)

Total flavonoids were measured using colorimetric assay developed by Zhishen *et al*., (1999). ` The total phenolic compounds were determined with Folin-Ciocalteu reagent according to the method described by Danial and George (1979). The results were expressed as milligrams of gallic acid equivalent per gram of dry extract weight.

Phenolic compounds

Phenolic compounds were fractionated and identified by HPLC according to the method of Goupy *et al.* (1999).The fractionation was conducted at Food Tech. Res. Inst. A.R.C., Giza, Egypt.

Microbiological methods

Total viable bacterial count, yeast and mold were determined according to APHA (1992) .The counts were calculated per one ml of sample as colonies formed unit (CFU).

Sensory evaluation

Sensory properties (color, taste, aroma and overall acceptability) of black mulberry juice and the nectar prepared from it were evaluated by the staff at Food Tech. Res. Inst., A.R.C. according to the method of Lindly *et al.* (1993).

Statistical analysis

The Statistical analysis of organoleptic evaluation was carried out using ANOVA under significant level of 0.05 for the analyzed data using SPSS (Ver. 11) .

RESULTS AND DISCUSSION

Physico-chemical parameter of fresh Black mulberry juice:

Based on the resulted data of Table (1), the juice yield of fresh juice was 62.80 %. The result was agreed with Amin and Attia (2003). Ercisli and Orhan (2008) who found that the fruit pulp were 64.03% and 68.58% of black mulberry genotypes.

Moisture content represented about 81.20 %. In this trend, Abo zaid (2014), found that the moisture content in fresh black mulberry juice was 80.18%.

Also, total soluble solids, pH value and tetratable acidity, (as malice acid), were 16.4%, 5.49 and 0.341% as malic acid, respectively, Table (1). These results are in accordance with Amin and Attia (2003).

Browning index measured by spectrophotometer at (420 nm) recorded 1.259, while the color value measured by Hunter lab recorded (L 27, a 8 and b 18), and the related parameter of juice clarity measured at (660 nm) was 35.9% respectively, Aramwit *et al* (2010) reported that the Color parameters of fresh black mulberry juice were (L 11.94 to 27.75, a 13.9 to 20.62 and the b value was 2.12 to 9.28) respectively. Also in Table (1), the viscosity of juice recorded 89.1 cp. The results are in accordance with obtained by Ercisli and Orhan (2007) and Editor *et al* (2013).

Table (1) Physico-chemical attributes of fresh Black mulberry juice.

Physico-chemical attributes	
Yield(%)	62.8
Moister (%)	81.20
Total soluble solids (%)	16.4
pH value	5.49
Color index at (420nm)	1.259
***Viscosity(cp)**	89.1
Clarity at (660 nm) %	35.9
***Colour parameters L**	27
a	8
b	18
Total sugars (%)	12.80
Reducing sugars (%)	11.20
Non-reducing sugars (%)	1.60
Protein (%)	1.62
Fibers (%)	2.20
L-Ascorbic acid (mg/100g)	28.22
Titratable acidity(% as malice cid)	0.341
Total antioxidant capacity%	53.72
Total flavonoids (mg/100g)	52.32
Total anthocyanin (mg/100g)	212.77

****Total phenol (mg GAE /100g)**	434.61

*Colour parameters L- (lightness darkness) a- (yellowness bluness) b- (redness greness)
**GAU- gallic acid equivalents

Concerning total sugars, reducing sugars and non- reducing sugars of fresh black mulberry juice, the analytical data represented 12.80 %, 11.20% and 1.6 % respectively, table (1). Neva and Memnune (2008) reported that the main sugars in fresh black mulberry juice were reducing sugars, which ranged between (9.42-15.46%) and non-reducing sugars ranged from 1.57- to 4.36% on fresh weight basis respectively. The determined protein and fiber of fresh black mulberry juice represented 1.62 % and 2.20 % respectively. These results are in accordance with obtained by Singhal *et al* (2010).

The percent of L-ascorbic acid of the fresh black mulberry juice was 28.22 mg/100g. This agree with Amin and Attia (2003), Ercisli and Orhan (2007) and Yildiz (2013).

As natural phytochemical components, for instance, total antioxidant capacity (%), total flavonoids, total anthocyanin (mg/100g) and total phenols, found of fresh black mulberry juice represented 53.72 %, 52.32 (mg/100g), 212.77 (mg/100g) and 434.61 (mg GAE /100g) respectively. These results are in agreement with Shih *et al* (2010) and Contessa *et al* (2013).

Identification of phenolic compounds using HPLC:

From Table (2) and Fig. (1), it could be notice that fourteen phenolic compounds among the seventeen authentic samples of standard were detected on mulberry juice. The detected compounds were, 3- hydroxyl-terosol ,oleuropein, catechol, benzoic, chlorogenic acid, salicylic acid, catchin, ellagic, caffeine, ferulic, caffic, gallic, vanilic and cenamic acid, which counted (9.414 mg/100g, 3.513, 2.878, 2.789, 23.75, 1.729,18.20, 1.605, 4.41, 4.30, 4.14, 2.68, 2.37 and 1.49 mg/100g respectively.

Table (2): Fractionations of phenolic compounds using HPLC method of fresh black mulberry juice.

Phenolic Compounds	**(%)**
Cinamic acid	1.494
Ellagic	1.6056
Ferulic	4.3006
Caffiien	4.4133
Catachin	18.200
Caffiic	4.1480
Salsalyic	1.72910
Chlorogenic	23.756
Gallic acid	2.688
Catechol	2.87810
3-hydroxy-tyrosol	9.414
Oleuropein	3.5130

Vanilic	2.376
Benzoic	2.789

Fig 1

Clarification processes of fresh juice:

Clarified mulberry juice as affected by using gelatin agent and UF precise

The main determined parameters used to know the efficiency of clarification process, clarity and viscosity. The percent of clarity increased from 35.9% of turbid juice to 75.5% in clarified juice, as a result of using gelatin agent, (Table 3). On the contrary, Table (3) also shows the decreasing of viscosity from 70 cp of turbid juice to 55 cp in clarified mulberry juice. These results were in agreement with those reported by (Siebert and Lynn., 1997) and (Kwang-Sup *et al*., 2004).

To explore the efficiency of UF process on resultant mulberry juice, TSS%, pH value, clarity and viscosity parameters were evaluated, Table (3). They were 16.1, 5.40, 97.5 and 10 cp in UF implemented juice comparing with turbid juice, which was 16.4, 5.49, 35.9 and 89.1 cp respectively, table (3). The results were agreement with (De-Barrod and peres. 2003), Rai *et al* (2005) and (Alvarez *et al*., 1998).

Effect of concentration process on some phsico-chemical attributes of reconstituted clarified mulberry juice

As implemented in the production line of some factories, after clarification processes, concentration process follows it. Also for analyzing the physico-chemical constituents as well as the sensory properties of the concentrates, it is a must to reconstitute the concentrates into the initial total soluble solids as found in the fresh juice, without treatment.

Dealing with the percent of clarity determined of clarified mulberry juice (by UF and gelatin),concentrated by rotary evaporator, it reached at zero time 69.8% compared with the fresh juice which was 35.9% and reached 93.7% in the case of UF applied juice. When freezing concentration used, slightly increasing was also observed of UF and gelatin implemented mulberry juice, (Table 4). The results of freezing concentration met with obtained by Askar *et al*., (1981).

Color index of fresh mulberry juice was also affected by concentration type used as well as the clarification type. In the case of freezing concentration, remarkable decrease of color index was observed than that concentrated by rotary evaporator. Indicating that the UF applied juice surpassed the gelatin treated juice of both rotary evaporator and freezing concentration method, (Table 4).

Small reduction of total sugars was observed during concentration conducted by freezing concentration followed by rotary evaporator and also when UF clarification and gelatin agent used. Dealing with reducing sugars of all application, the same trend was observed, Table (4).

Incubation time, temperature, chemical agent, nature of UF membrane, Concentration method and, pasteurization affect ascorbic acid stability. As shown in Table (4), when concentration applied by rotary evaporator, ascorbic acid decreased from 28.22 mg/100g of fresh turbid juice to 10.33 mg/100g of gelatin treated and 11.11 mg/100g of UF treated juice. While when concentration applied by freezing, ascorbic acid decreased from 28.22 mg/100g of fresh turbid juice to 15.18 mg/100g of gelatin treated and 17.22 mg/100g of UF treated juice, table (4). The results were in accordance with those reported by, Butera *et al* (2002), and Cassano *et al* (2003).

Total flavonoids (mg/100g), total anthocyanin (mg/100g), total phenol mg GAE/g and total antioxidant capacity of mulberry juices were listed in table (4). Generally there was observing decreasing of previous components as affected by clarification and concentration method used.

The UF applied juice surpassed gelatin treated, and also freezing concentration surpassed the concentration by rotary evaporator. Based on the previous data discussed, freezing concentration was chosen, and used during storage period.

Table (3): Effect of clarification by gelatin agent and UF on some parameters of clarified black mulberry juice

Parameter	fresh	Gelatin	(UF)
Total soluble solids (%)	16.4	16.6	16.1
PH value	5.49	5.45	5.40
Clarity at (660 nm)	35.9	75.5	97.5
***Viscosity(cp)**	89.1	55	10

*Cp- centipoise

Table (4): Some physio-chemical attributes of clarified black mulberry juice as affected by concentration using rotary evaporator

Juice / properties	fresh	Black mulberry concentration process by rotary and freezing concentration			
		rotary		freezing concentration	
		gelatin	UF treated	gelatin	UF treated
Total soluble solids (%)	16.4	16.4	16.4	16.4	16.4
PH value	5.49	4.64	4.60	4.84	4.80
Colour index at (420nm)	1.259	1.440	1.411	1.380	1.355
Clarity at (660 nm) %	35.9	69.8	93.7	74.30	96.90
Total sugars (%)	12.80	11.97	11.75	12.11	12.55
Reducing sugars (%)	11.20	10.33	10.19	10.44	11.31
Non-reducing sugars (%)	1.60	1.64	1.56	1.67	1.24
Titratable acidity % (as citric acid)	0.341	0.444	0.431	0.450	0.443
L-Ascorbic acid (mg/100g)	28.22	10.29	11.11	15.18	17.22
Total antioxidant capacity %	53.72	42.31	45.39	48.11	49.25
Total flavonoids (mg/g)	52.32	41.79	40.92	47.23	48.17
Total anthocyanin (ug/100g)	212.77	135.19	148.20	162.29	168.88
***Total phenol (mg GAE /g 100 g)**	434.61	395.1	409.3	411.15	422.22

***** GAU- gallic acid equivalents

Sensory properties of reconstituted juice at zero time:

Sensory properties of all reconstituted clarified juices at zero time were illustrated of Table (5). From the analyzed data, it can be concluded that the reconstituted clarified juices at zero time were accepted in their sensory attribute such as color, taste, aroma and overall acceptability, which ranged between 7.7 and 9.8 degrees.

Table (5): Organoleptic evaluation of reconstituted mulberry juice.

Parameter	Gelatin concentration basket centrifuge	Ultrafiltration concentration basket centrifuge	Gelatin concentration rotary	Ultrafiltration concentration rotary
Color	8.6^{c}	9.5^{a}	8.1^{de}	9.1^{ab}
Taste	9.6^{a}	8.8^{bc}	8.6^{c}	7.7^{d}
Aroma	9.8^{a}	8.8^{bc}	9.1^{b}	7.9^{c}
Overall-acceptability	9.6^{a}	9.4^{ab}	8.5^{b}	7.8^{c}

Means in the same raw with different letters are significantly different (pp≤0.05)

Effect of storage on Phsico-chemical attributes of clarified reconstituted mulberry juice.

The effect of storage conditions on color index at 420 nm of reconstituted clarified mulberry juice by gelatin agent was observed of Table (6), and the data revealed that the color index increased from 1.38 at zero time to 1.52 at the end of storage period. While in the case of UF, the color index reached 1.512 at the end of storage period, Table (7).

Concerning, clarity at 660 nm determined, which shows of table (6 and 7), gradually decreasing during storage period was observed of both gelatin treated and also UF treated juice.

Also, there were some what changed of acidity, which recorded little increasing during storage period. On contrary pH value was decreased gradually during storage period, of gelatin treated and also UF treated juice table, (6 and 7).

Dealing with total sugars and reducing sugars of reconstituted black mulberry juice, there was little decreasing during storage period of gelatin treated and also UF treated juice Table, (6 and 7).

The effect of storage conditions on color index at 420 nm of reconstituted clarified mulberry juice by gelatin agent and UF was illustrated in table (6 and 7), and the results show that the reconstituted juice at zero time contained 15.18 mg/100g and17.22 mg/100g, whereas, they were 6.52 mg/100g and 8.36 mg/100g of gelatin agent and UF treated juice. The results were in agreement with (Davey *et al*., 2000 and Franke *et al*., 2004). Total antioxidant capacity as mg/100g , total flavonoids mg/100g, total anthocyanin as µg/100g and the total phenol GAE/g as natural antioxidant agents of reconstituted black mulberry juice were determined and the data revealed that, there were gradually decreasing of them during storage period at room temperature. This is agree with Polydera *et al* (2004), and Kalt *et al* (1999).

Microbiological aspects of black mulberry juice:

Some of basic microbial analysis of fresh, zero time and after 4 months of

storage period such as(total bacterial count and yeast & mold counts) of all mulberry juice samples were analyzed, (Table 8) .

The percent of total counts resulted from analyzed data of gelatin and UF treated juice ranged from 1.0×102 cfu/g to 3.2×102 cfu/g. whereas the yeast & mold counts ranged from 2.8×102 cfu/g to 4.6×102 cfu/g. This means the reconstituted mulberry juice at zero time and after 4 months of storage period are safe.

This could be attributed mainly to the very low pH observed (3.94- 4.04). In addition, conditions under which the juice was processed, stored, and/or served might have contributed to the betterment of the product. In fact, its low pH did not inhibit the growth of acid tolerant yeasts and molds, and this allowed their proliferation to level as high as 3.5 x 104 and 4.0 x 104 log CFU/ml, respectively Olorunjuwon *et al*., (2014).

Organoleptic characteristics of mulberry nectar:

Mulberry nectar prepared from its concentrate was sensory evaluated. The statistical analyzed sensory data of Table (9), declared that the whole parameters were accepted and ranged from 7.5 to 8.8 degree. The sensory properties of mulberry nectar indicated that the prepared nectar from its concentrate after the end of storage is accepted and met with the previous evaluated analysis.

Table (6): Effect of storage of concentrated clarified (by gelatin agent) mulberry juice, on physico-chemical attributes.

Parameter	Black mulberry					
	fresh	**Zero time**	**1 month**	**2 month**	**3 month**	**4 month**
pH value	5.49	4.84	4.78	4.71	4.66	4.58
Color index at (420nm)	1.259	1.380	1.410	1.460	1.511	1.522
Clarity at (660 nm)	35.9	74.30	74.0	73.6	69.1	60.36
Total sugars (%)	12.80	12.11	12.98	12.78	12.66	12.61
Reducing sugars (%)	11.20	10.44	11.17	11.02	10.87	10.79
Non-reducing sugars (%)	1.6	1.67	1.28	1.76	1.79	1.85
Titratable acidity% (as citric acid)	0.341	0.450	0.462	0.477	0.484	0.492
L-Ascorbic acid (mg/100g)	28.22	15.18	13.22	10.39	8.96	6.52
Total antioxidant capacity %	53.73	48.11	46.33	44.21	38.36	35.49
Total flavonoids (mg/g)	52.32	47.23	45.66	42.39	38.11	35.33
Total anthocyanin (ug/100g)	212.77	162.29	148.39	132.11	121.21	118.85

***Total phenol (mg GAE /g)**	434.61	411.15	398.33	381.02	364.65	357.25

*GAU- gallic acid equivalents

Table (7): Effect of storage of concentrated mulberry juice, clarified by UF on physico-chemical constituents.

Parameter	Black mulberry					
	fresh	**Zero** time	**1 month**	**2 month**	**3 month**	**4 month**
pH value	5.49	4.80	4.72	4.64	4.58	4.44
Colour index at (420nm)	1.259	1.355	1.411	1.489	1.496	1.512
Clarity at (660 nm)	35.9	96.9	94.36	91.96	89.63	87.98
Total sugars (%)	12.80	12.55	12.10	11.88	11.80	11.71
Reducing sugars (%)	11.20	11.31	11.14	11.01	10.96	10.84
Non-reducing sugars (%)	1.6	1.24	0.96	0.78	0.84	0.86
Titratable acidity % (as citric acid)	0.341	0.443	0.471	0.510	0.533	0.541
L-Ascorbic acid (mg/100g)	28.22	17.22	14.20	12.11	10.91	8.36
Total antioxidant capacity %	53.73	49.25	47.33	45.10	41.32	39.39
Total flavonoids (mg/g)	52.32	48.17	47.36	45.30	41.44	40.05
Total anthocyanin (ug/100g)	212.77	168.88	144.36	139.38	131.27	128.28
***Total phenol (mg GAE /g)**	434.61	422.22	414.33	401.12	384.92	371.25

Table (8): Microbiological counts of reconstituted clarified(by gelatin) black mulberry juice as affected by storage conditions.

UF treated Black mulberry juice / **storage period**	**Counts *(CFU)**			
	gelatin		UF	
	Total count	**Yeast & mold**	**Total count**	**Yeast & mold**

Fresh	3.2×10^2	4.6×10^2	3.2×10^2	4.6×10^2
Zero time	1.3×10^2	2.8×10^2	1.1×10^2	3.1×10^2
After 4 month	1.1×10^2	3.0×10^2	1.0×10^2	3.3×10^2

Table (9): Sensory properties of mulberry nectar.

Parameter	Gelatin concentrated by basket centrifuge	UF concentrated by basket centrifuge
Color	8.3^b	8.7^a
Taste	8.5^a	8.1^b
Aroma	8.7^a	8.2^b
Overall- Acceptability	8.4^a	8.0^b

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دراسات كيميائية و تكنولوجية على عصير و مركز التوت

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الملخص العربى

تعتبر عصائر الخضر والفاكهه سواء كانت فى صوره رائقه او غير رائقه والمنتجات التى تصنع من خلالها مثل المركزات و الشراب المركز والنكتار والمشروبات المتعدده التركيز ذات قيمة غذائيه و صحية وإقتصاديه بالغه الاهمية .

فيما يخص التركيب الطبيعى والكيماوى لعصير التوت الاسود ،فمن خلال النتائج وجد انه يحتوى على نسبه تصافى وصلت الى ٦٢.٨% ومحتوى من الرطوبه وصل الى ٨١.٢٠% .كما سجلت قيم المواد الصلبة الكلية الذائبة و القم الهيدروجينى و الحموضة الكلية (١٦.٤% و٥.٤٩ و ٣٤١.% على التوالى.اما فيما يخص التلون البنى فقد كان ١.٢٥٩ نانوميتر. أما الشفافية فقد كانت ٣٥.٩%. ومن خلال النتائج ايضا فقد سجلت السكريات الكلية اعلى القيم بالنسبة لباقى المركبات ، حيث كانت ١٢.٨% فى حين وصلت السكريات المختزلة إلى ١١.٢٠%. كما إحتوى العصير الطازج على ٢٨.٢٢ملجم/١٠٠جم من حمض الاسكوربيك و ٥٣.٧٢ ملجم/١٠٠جم من مضادات الاكسدة الكلية. كما إحتوى العصير على ٥٢.٣٢ مجم/١٠٠جم من الفلافونويدات و ٢١٢.٧٧ ملجم/١٠٠جم من الانثوثيانين ، علاوة على إحتوائه نسبة مرتفعة من الفينولات الكلية ، حيث وصلت إلى ٤٣٤.٦١ ملجم من حامض الجاليك/ جم من المستخلص الجاف.

اما فيما يخص الركبات الفينولية المقدرة بالفصل الكروماتوجرافى ، تم الحصول على ١٤ مركبا فينوليا.

كما أكدت النتائج ان الترويق بالترشيح الدقيق قد تميز عن الترشيح بإستخدام الجيلاتين.

و فيما يتعلق بنتائج تاثير عمليه التركيز على الخواص الطبيعيه والكيماويه ،اكدت ان التركيز بالتجميد اعطى خواص جيده للعصير الناتج,كما اكد ذالك اختبار التقييم الحسى عقب التركيز مباشره. ومن خلال النتائج اثناء تخزين العصير المركز بالتجميد على درجه حراره الغرفه ٢٥+٥م , وجد ان مكونات العصير الرائق بالترشيح الدقيق كانت اكثر تميزا وخاصه فى خصائص الترويق عنه فى العصير الناتج بالترشيح بالجيلاتين، علاوة على ان العصير عامة أمن ميكروبيا.

وفى النهايه تم اجراء تقييم حسى للنكتار المصنع من العصير المركز بعد انتهاء فتره التخزين وهى اربع شهور حيث اكدت النتائج ان النكتار الناتج في كلتا الحالتين كان مقبولا لدى المحكمين وتتماشى نتائجه مع النتائج السابقه للتحليل الطبيعى والكيماوى.

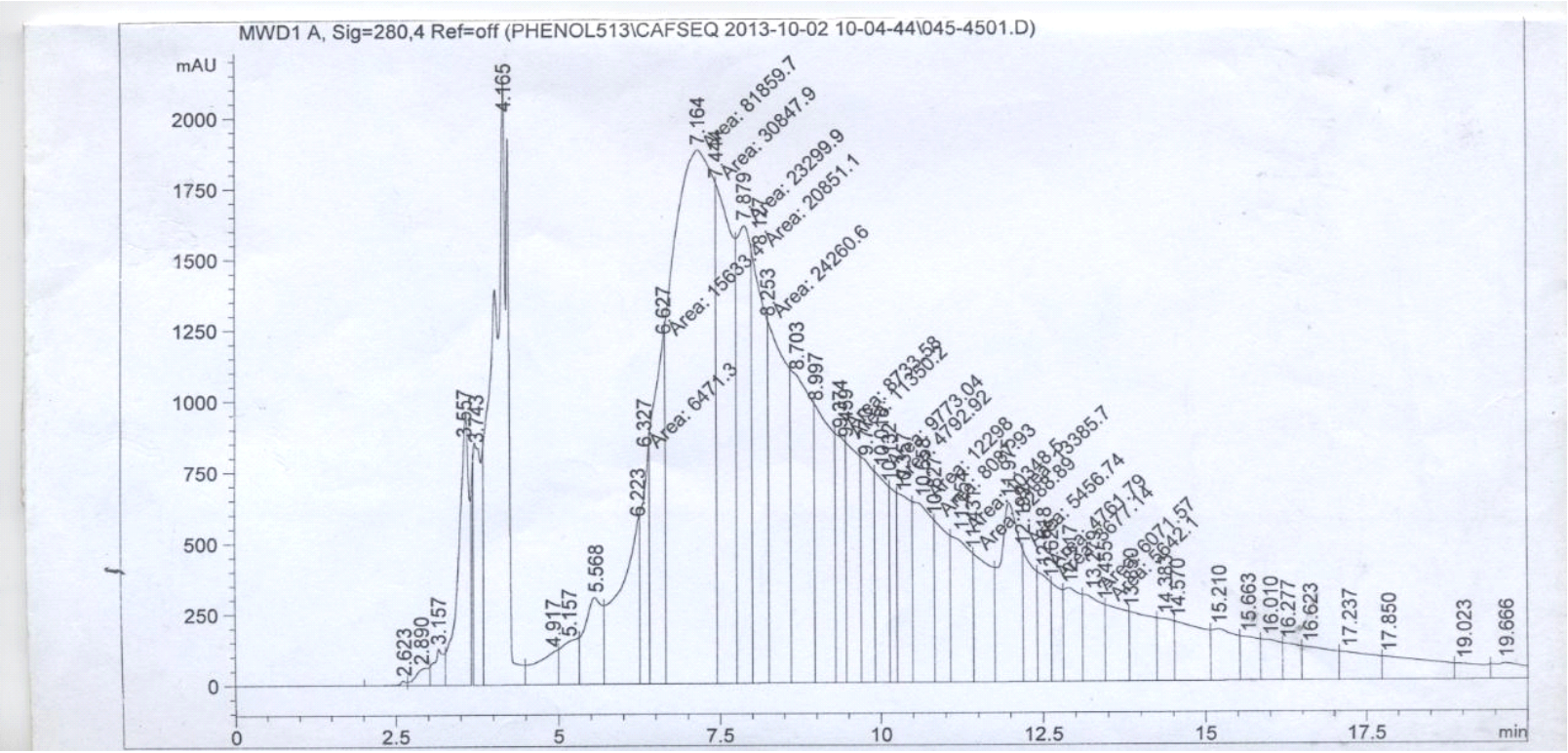

Fig (1): HPLC chromatogram of phenolic compounds of fresh mulberry juice.