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The Effect of Chemical Treatments and Condensation Technique in Biologically Active Substances in Grape Molasses

Mohamad Anas AL-Rouz[1], Tahla, M. K.[1], Hamed, F.[2]

[1]Food Sciences Dept., Faculty of Agriculture, Damascus University, Syria
[2]Food Science Section in General Commission of Scientific Agriculture Research Center, Syria

Abstract: This research was conducted at the Faculty of Agriculture, Damascus University, Syria in the period (2012 - 2013), to study the effect of the industrial methods and chemical treatments on the chemical structure, biologically effective compounds and antioxidant activity in grape molasses. The results reveal that the suggested method by adding 2% of the limestone to the grape juice during the manufacturing process and using the vacuum technique, has proved a highly effective and significant differences comparing with the traditional method in improving the quality of grape molasses, which contributed to the reduction in moisture and increase of the Total Soluble Solids and Total Sugars. Also worked to reduce the loss of vitamin C and total phenols, thus maintained a high percentage of antioxidant activity in molasses.
Key words: grape molasses, vitamin C, total phenols, antioxidant activity.

Introduction.

Grapes in Syria are considered of the most important agricultural economic products and occupy an excellent rank among the fruit species locally produced, which are significantly desired and required from all segments of the community. Grapes growing spread in different provinces of the country, especially Damascus, Al Sweida, Homs, AL Quneitra, Hama, Idlib, Latakia and Aleppo (1). The cultivation of the vine declines in Syria in recent years. This declination has been observed to be out of the reduction of the cultivated area of vine of 69288 hectares in 2000 to 4,500 hectares in 2012, the thing that led to the lessening of the production of 409 450 tons to 325,000 tons (2)

The nutritional as well as economic importance of the vine tree is due to its production of a high-nutritional value fruit as its fruit is used either as ready to eat grapes (table grapes) which are eaten fresh or as dried grapes (raisins) or grapes made in the form of jams, molasses and vinegar. Juices and alcoholic beverages are also made from these fruits. (3) However, this fruit is also important because of oil extracted from the seeds of grapes that is used in some chemical industries (soap, dyes). The leaves of the fruit are also cooked and used as food for humans or as feeds to the animal after being dried. Moreover, some pharmaceuticals which are useful for blood circulation in the human body are extracted from the grapes' dregs.

Dregs are also used as feeds for animals or in the preparation of some organic fertilizers after being stripped of seeds. Vine tree is also used as a decoration tree in the form of arbors. In addition to the high nutritional value the fruits of grapes and raisins contain, they are also of a high therapeutic and healthy value. (1)

Grapes are classified into four groups: table grapes, wine grapes, raisins grapes, and juice grapes (4). They are also categorized in a number of local varieties as Hamid et al stated (3), most importantly: Zini, Local, confectionary, Red Dumaini, Red Dirani, Black Dirani, White Drpley, Mariami.

Grape fruits contain sugars (glucose and fructose), organic acids, mineral salts, vitamins and enzymes, Table (1). The percentage of tannic substances in red grapes' crust is up to 6-3% (5).

Table (1). The average of the rates of some main ingredients of fresh grape fruits in 100g of the wet grapes.

Water	Ash	sugars	Organic acids	protein	fat	Remaining substances
65-85	0.3-1	15-30	0.3-1.5	0.9-0.15	1	0.3-1

The biologically active materials in grapes:

The fruits of grapes, the black apricot and Berries are considered important sources of antioxidants that are important to human health, as these compounds possess anti-inflammatory, bacteria and viruses. Moreover, these antioxidants possess the effects of regulating blood pressure (2), in addition to their tonic effect on the cell (4)

As mentioned by Syvacy and Sokmen (6), Grape types are considered as natural sources of antioxidants, the most important of which include phenolic compounds (flavonoids and Alonthusianinat) and vitamin C.

Vitamin C is a soluble vitamin in water and very sensitive to heat, light and oxygen (7). It is significantly featured for its acidic as well as returned features. Vitamin C is also one of the important nutrients as it is necessary for the synthesis of collagen, and helps maintain the skin, gums and blood vessels, and lowers cholesterol level in the blood plasma (8). The foods rich in vitamin C reduce the risk of developing the gastrointestinal gut cancer as well as they strengthen the immune system.

Free radicals and antioxidants:

Free radicals are chemical species (atoms, molecules) which contain a solo electron, not double. This solo electron makes the atom unstable; it makes it (atom) a free radical which if not curbed, it would generate other free radicals, and would cause damage to the surrounding cells and tissues (9). Because of the high effectiveness of the free radicals they interact with biomolecules in the body snatching their electrons and thus causing their oxidation. Thus, these molecules become ineffective in the cell and many of the cellular components are damaged due to the active oxygen types.

However, proteins, lipids, sugars and nucleic acids are the most affected. The result of this damage is various illnesses as Arteriosclerosis and cancers (9; 2). Antioxidants are defined as compounds which are capable of preventing or hindering the oxidation of other compounds when they are of few concentrations as they submit an electron to the free radicals and oxidize in turn to become weak non-toxic free radicals, simply because they are not effective concerning their fastness by buzzing (10). Antioxidants contribute to the mitigation or prevention of many diseases that affect the human body as Arteriosclerosis, arthritis, injuries arising from the re- perfusion of the tissues, damage to the central nervous system, stomach inflammation, cancer and AIDS (11)

Molasses industry spreads in many countries producing grapes as Syria, Turkey and Iran. It also spreads widely as a homemade in many regions of the Syrian Arab Republic, especially in Damascus Rural, and AL Sweida. Some producers use it to meet domestic consumption and the needs of the local markets of this material. Molasses is considered a popular food that is useful and delicious. It is one of the traditional products which Syria is famous for. It is an inherited industry from ancestors to descendants.

As It depends on personal experience and individual knowledge, this industry remained the preserve of a limited number of families for a long time (12). This traditional industry began to gradually engage modern scientific bases upon the scientific progress in all fields (8). Despite the distinctive unique of this traditional industry and its link, especially to the Levant, it proves to spread in a variety of countries such as Turkey, Iran and Iraq, more specifically, molasses industry of dates (12). Molasses types vary according to the type of grape and the degree of sweetness, as well as according to the intensifying degrees. Molasses industry is made according to the traditional way as follows (1).

Number of molasses factories in Syria have reached 124 factories in 2006 and declined in 2007-2008 to 110 factories in 2009 to 89 factories. In 2010, the Annual Agricultural Statistical Abstract in 2010 became 98 factories.

Research objectives

Given the importance of molasses as a traditional nutritional product in Syria, this research has been conducted in order to identify the ideal production conditions to get molasses of high-quality and financial benefit. Moreover, this research investigates the influence of the condensation technique in both traditional as well as suggestive methods in some of the biologically active substances of the produced molasses which are naturally existent in the fruits of grapes.

Materials and methods

The raw material:

Grape has been selected of the Salti type that is used in Syria to make molasses. It is used at 10 kg for each treatment as a raw material for the research. Salti grape is considered of the most significant produced types. It constitutes 20% of the total production (14), and is mainly used in molasses manufacturing.

Technical operations:

The technical processes on the fruit grape for the production of molasses have been done according to the following stages.

Getting grape juice

Grape fruit juice has been taken from the whole fruit after being washed through mashing.

Adding lime:

Pure Lime was added (calcareous soil) purity of 94% with a percentage of (1-2 %), and after this, the grape juice was filtered by using a normal stainless steel refinery.

The condensation of the grape juice has been conducted in both the traditional and the suggestive methods.

The traditional method:

Grape juice has been condensed under atmospheric pressure (open cooking). This is a commonly used method in the food industry and mainly in many of the molasses mills, where juice is condensed up to soluble solids ratio of about (68 ± 1)%.

The Modern method:

Grape juice has been condensed under vacuum using a rotary evaporator (rotary evaporator), where juice has been condensed in the device flask 55 m° until getting whole soluble solids about (68+-1)%.

Packing and keeping molasses output :

Produced Molasses has been maintained in glass bottles in the fridge until being investigated.

1- Designate biologically active substances :

The biologically active substances of both grape juice and the final product (molasses) have been designated.

Ascorbic acid amount estimation (Vitamin C - Ascorbic Acid):

The expression of ascorbic acid content using a calibration method with dye 6.2 dual -chlorophenol Endo phenol that oxidizes ascorbic acid to become hydrogen- skimmed ascorbic acid, by (15).

Estimating the total phenols:

Total phenols were extracted from the investigated samples according to the method (6) with some modifications. 10g of the sample was taken by a polyethylene tube of 50 ML capacity. Then 30 ml of released ethanol was added. Next, phenols were extracted by using a magnetic engine at maximum speed and at the room temperature for one hour. After this, the sample was weighed by a Centrifuges laboratory device at (3000 rpm) speed, for 5 minutes and then a serene liquid for analysis was taken.

Phenols have been quantified by using the Ciocalteu –Folin method used by (16) with some modification. These modifications are: 2 ml of the previously prepared sample have been taken, and then 3 ml of distilled water 0.2 ml of detector Volyn are added then put in a volumetric standardized flask with 10 ml capacity. The mixture is shaken by the pipe engine to extend in two minutes in room temperature. Then, 4 ml of Na_2CO_3 7% are added. The size is completed by adding the distilled water until the completion of the size of 10 ml. The former mixture is mixed and left for two hours at room temperature. After this, weighing and the measurement of the floating by Spectroscope Alsoia at the wavelength of 750 nm is done. Tannic acid is used as a returned standardized solution to prepare the calibration curve, a concentration range of 0-100 micrograms / mL

Antioxidant Activity Assay.

The anti -oxidant activity measurement has been done by appointing the curbing free radicals' activity according to the free radical method 1.1-diphenyl -2-picryl – hydrazyl(DPPhH) according to Singh et al.(17) as following.

The same volume of solution (DPPH) (65 micromoles in methanol)is added to alcoholic sample extract (1 g sample in 100 ml methanol). After mixing the above mentioned mixture by the tube (vortex), its absorption is measured at the wavelength of 517 nm after 30 minutes. Methanol is used in the experiment instead of the sample.

An experiment is conducted on BHT and ascorbic acid (50 mg / liter) to be compared with the results given by the extract's samples. The curbing activity of free radicals is demonstrated by calculating the percentage of inhibition of oxidation out from the equation: % Inhibition = [(A- A ') / A] X100.

A: The Primary absorbance of the control at 517 nm.

A': The final absorbance of the sample tested at 517 nm.

2- Statistical analysis:

Statistical analysis of the data was carried out depending on the design and random pieces by three replicates per treatment. Analysis of variance and finding a less LSD difference between the averages of the treatments at the level of statistical evidence has been used 0.05. For this statistical program, 18 SPSS is used.

Results and Discussion:

Investigating the effect of the condensation technique in some of the biologically active substances of the produced grape molasses .

1-The effect of the condensation technique in vitamin C:

The results ,(Table 2), show a difference in the average vitamin C content in the produced grape molasses upon the different manufacturing treatments, where the existence of this contrast between the testified condensation technique and in the comparison with the raw grape juice was noticed (the control).

The difference in the proportion of the added limestone soil interacting with the condensation technique (atmospheric pressure - under vacuum) results the existence of qualitative differences in the average amount of vitamin C in the molasses produced, table 2. We notice that the qualitative value of F test was smaller than the level of the statistical evidence (0.05). Grapes manufacturing process led to a qualitative loss of vitamin C compared to grape juice. The results show the superiority of treatment (soil calcareous 2%, under vacuum) due to its effect on the amount of vitamin C, and qualitative differences from the rest of the treatments. The average amount of vitamin reached C 1.08 mg \ L, followed by treatment (soil calcareous 1%, under vacuum) and reached the amount of vitamin C 1.06 mg \ L. Thus, the use of the modern condensation method under vacuum and with interaction with the increase in limestone soil 2%, led to maintaining a qualitative amount of vitamin C in produced molasses and therefore Getting best productivity qualifications. The results agrees with (18).

Table 2: This table shows the amount of vitamin C in grape molasses according to the joined interaction of the adding average and the condensation technique.

Average of vitamin C mlg\L		Technique	The Limestone percentage %
± SE	Mean		
10.50	22.67 a*	**Raw grape juice (control)**	**0**
0.16	0.19 c	**Atmospheric pressure**	**1**
0.05	1.06 b	**Under vacuum**	
0.10	0.22 c	**Atmospheric pressure**	**2**
0.09	1.08 b	**Under vacuum**	
0.008		**F**	
0.001		**Sig.**	
0.620		**LSD**	

-The different vertical letters refer to qualitative differences among the averages on the level of the statistical evidence 0.05.

1-The effect of the condensation technique in total phenols .

The results, (Table 3), show a difference in total phenols' averages in produced grape molasses according to different manufacturing treatments. It was noticed that there is a difference between the two techniques of the testified condensation and also in comparison with raw grape juice (control).

The difference in the proportion of the added Limestone soil interacting with the condensation technique (atmospheric pressure - under vacuum) results the existence of qualitative differences in the average amount of total phenols in the produced molasses, table 3. We notice that the qualitative value of F test was smaller than the level of the statistical evidence (0.05). Grapes manufacturing process led to a qualitative loss of total phenols compared to grape juice. The results show the superiority of treatment (soil calcareous 2%, under vacuum) due to its effect on the amount of total phenols, and qualitative differences from the rest of the treatments. The average amount of total phenols reached C 1.12 mg \ L, followed by treatment (soil calcareous 1%, under vacuum) and reached the amount of total phenols 1.01 mg \ L. Thus, the use of the modern condensation method under vacuum with interaction with the increase in limestone soil 2%, led to maintaining a qualitative amount of total phenols in produced molasses and therefore getting best productivity qualifications. These results confirmed these found by (6)

Table 3: This table shows the amount of total phenols in grape molasses according to the joined interaction of the adding average and the condensation technique.

Average of total phenols mlg\L		technique	The Limestone percentage %
± SE	Mean		
6.41	16.01 a*	**Raw grape juice (control)**	**0**
0.03	0.21 c	**Atmospheric pressure**	**1**
0.03	1.01 b	**Under vacuum**	
0.20	0.35 c	**Atmospheric pressure**	**2**
0.06	1.12 b	**Under vacuum**	
0.067		**F**	
0.800		**Sig.**	
0.643		**LSD**	

-The different vertical letters refer to qualitative differences among the averages on the level of the statistical evidence 0.05.

1-The effect of the condensation technique in the anti-oxidant activity:

The results show a difference in anti-oxidant's averages in produced grape molasses according to different manufacturing treatments. It was noticed that there is a difference between the two techniques of the testified condensation and also in comparison with raw grape juice (control).

The difference in the proportion of the added limestone soil interacting with the condensation technique (atmospheric pressure - under vacuum) results the existence of qualitative differences in the average amount of anti-oxidant activity in the produced molasses, table 4. We notice that the qualitative value of F test was smaller than the level of the statistical evidence (0.05). Grapes manufacturing process led to a qualitative loss of anti-oxidant activity compared to grape juice. The results show the superiority of treatment (soil calcareous 2%, under vacuum) due to its effect on the amount of anti-oxidant activity, and qualitative differences from the rest of the treatments. The average amount of anti-oxidant activity reached 60.68 and 60.16 consecutively. Thus, the use of the modern condensation method under vacuum and with interaction with the increase in limestone soil 1 and 2%, led to maintaining a qualitative amount of anti-oxidant activity in produced molasses and therefore getting best productivity qualifications.

This decrease in the anti-oxidant activity is due to the loss of many of the active compounds out of the high temperature of the manufacturing such as the loss of vitamin C. These results agree with what have been proved by (18), (19), and (20). Vitamin C is of a high activity in curbing the free radicals and contributes to the anti- oxidant activity. Moreover, the difference is also because of the loss in the whole phenols as emphasized by (21) on the existence of a linear correlation between the total phenols and the antioxidant activity that is estimated by a method which returns iron (FRAP).

Table 4: This table shows the amount of total phenols in grape molasses according to the joined interaction of the adding average and the condensation technique.

Average of anti-oxidant activity		technique	The Limestone percentage %
± SE	Mean		
0.01	80.79 a*	**Raw grape juice control**	**0**
0.42	54.35 c	**Atmospheric pressure**	**1**
0.62	60.16 b	**Under vacuum**	
0.51	55.12 c	**Atmospheric pressure**	**2**
0.24	60.68 b	**Under vacuum**	
124.807		**F**	
0.000		**Sig.**	
1.751		**LSD**	

-The different vertical letters refer to qualitative differences among the averages on the level of the statistical evidence 0.05.

Conclusions and recommendations

We find that the suggestive method which has been done by adding 2% limestone soil to the grape juice during the manufacturing process, using the manufacturing method under vacuum, has proved highly effective and qualitative differences in improving the produced molasses' qualities compared to the traditional container method. This method has contributed to reducing the loss in the amount of vitamin c and the whole phenols and thus maintaining a high percentage of the anti- oxidant activity.

It is necessary to study some of other variables such as industrialization heat temperature in order to find the best degrees that are suitable for condensation, a process by which conserving the largest possible amount of vitamin C, total phenols and other temperature sensitive compounds is achieved. Thus, improving the content of the produced molasses of active compounds and anti dioxide action is guaranteed.

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