The Chemical Changes That Associating to Concentrates of Apple Juice during Storage Periods

Dia Alomar * Abd Alwahab Meree Anwar Alhaj Ali
Department. Of Food Science. Faculty of Agriculture . Damascus University .Syria

Abstract
we took in this work Twenty kg of apple juice concentrate (70%) from company of Natural Aljabal Juice from AL-suidaa Governorate and prarerd two concentarate (15% and 35%) by using distilled –sterlization water . these samples stoered at (4-20-30-40)ºc for 6 months . Chemical tests were assayed in the every month average two repeats to every concentrate and all different temperatures and recored the average, The chemical analysis results show: There is reduce in total soluble solids TSS , afew changes of pH and the acidity relaise in all samples that storage at 20 ºc beacause it is agreeable for oraganisms to grow.The produces of oeganisms were (patuline - diacytle- alcohol): the amount of patuline that is determine with Liquid Chromatography (HPLC) was less than 50micro gram/kg in juice in all samples and under all condtation storage that is less than limit value for fumble device, there was relaised of amount in diacytle in samples of juice 15% (4.1±0.3) ppm in the end of second storage at temperature 20 ºc while the amount of apple juice 35%-70% was very little 1.9ppm at temperature 30 ºc for apple juice in the third month of storage and 1.69 ppm for apple juice 70% in the third month of storage at 20 ºc, amonnt of alcohol relies to upper value (2.55 ± 0.1) % in apple juice 35% at 20 ºc in the sixth month of storage , the amount of alcohol in apple juice 20% was (1.25 ±0.4)% in the sixth month of storage and 0.52 % in apple juice 70% at 30ºc in the second month of storage .
Keywords: Apple juice concentrate - total soluble solids TSS- diacytle – patulin- alcohol.

1. Introduction

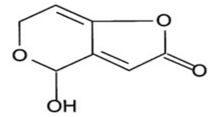
Is considered the most vulnerable to contamination by yeasts , molds and lactic acid bacteria apple juice and that more cases of contamination juices are bacteria-loving acidity and high temperature, and most of the existing microorganisms in the juice of the races that have the ability to withstand concentrations of sugary high (Alosmovilah) Being contain a sufficient amount of sugar and ranging acidity between 0.20 -1.7% and pH between 3.3 to 8.3, and when it is stored at different temperatures is exposed to several changes such as alcohol oxidation ,Alcoholic fermentation and fermentation organic acids by activity of yeasts and molds and bacteria .Raider in Composition of the juice in terms of the concentration of Total soluble solids solids, pH, acidity and form new compounds were not been in the juice such as diacetyl ($C_4H_6O_2$) , patulin ($C_7H_6O_4$) and Alcohol (C_2H_5OH) . [4] Pointed when compared to the growth of some microbial strains such as fungi : *Aspergillus niger Penicillum notatum* and yeasts: *Sacchromyces cerevisiae* and bacteria: *Lactobacillus plantaruium*, *Leuconostoc mesenteroides* in apples juices concentrations (30%) and (68%) during the storage period (3 months) to the emergence of variation in growth since the good growth in the center position concentration (30%), while in the other zero, concentration, and concluded from that that these microbial strains do not belong to Alosmovilah Microbiology.

Apple juice damage the formation of a binary compound Diacetyl by lactic acid bacteria comes, which is an organic compound commonly Butanedione his name (2.3) and outlined his formula $C_4H_6O_2$ chemical formula CH_3-CO-CO-CH_3.[1]

The more Ahtwae patulin on apple juice drink is patulin secondary metabolic output for various types of aerobic microscopic fungi (*Penicillum patulum - P.urticae - P.ctaviforme - P.expansum - Aspergillus clavatus - Byssochlamysspp - Penicillum notatum- Mucor- Rhizopus*) and mainly *Penicillium expansum* and *Penicillium patulum*, which are produced under special humidity and temperature conditions, and moves with many foods, especially apples-to-human, where secreted mushroom *P.expansum* patulin which causes a range of health effects, [2.] Reflected the acute symptoms of patulin occurrence of disorder ,spasm, congestion , edema pulmonary congestion with blood , swell the airways, circulation , intestinal bleeding and ulcers, so it has identified the European Union EU to focus the maximum limit of patulin in food by 50 mcg / L, and identified the US Food and Drug Administration USFDA per juices apples and concentrates apple vinegar with 50 mcg / l, but many countries have reduced this ratio to 35-25)) mcg / l due to the high rate of consumption of apple juice. [3] The

[1] CODEX ALIMENTARIUS COMMISSION.,1999- Joint FAO/WHO Food Standards Programme, Codex Committee on Food Additives and Contaminants Thirty-first Session, The Hague, The Netherlands, 22-29 March 1999, Position Paper on Patulin Prepared by France, Agenda Item 14(c), CX/FAC 99/16.
[2] MONICA, C, LUMINIŢA, C, GABRIELA, L, MIOARA, N, ENUŢA, NASTASIA, B, AND ALINA, B. I.,2011- Determination of patulin in apple juice .Romanian Journal of Food Science, 1(1): 65 –69.
[3] HIGUCHI, T., HAYASHI H., AND ABE K., 1997- Exchange of glutamate and gamma-aminobutyrate in a Lactobacillus

World Health Organization has also identified WHO Great daily dose to 0.4 mcg / kg of weight body / day [1] patulin has the following chemical formula:



$C_7H_6O_4$ (4-hydroxy-4H-furo [3, 2-c] pyran-2 (6H) –one

The patulin is lactone soluble in water, ethanol, acetone and acetate ethyle, ether and chloroform, but is not soluble in gasoline and ether petroleum, and there are as solid colorless crystals or white, and its molecular weight is 154.1 g / mol, and its melting temperature is (112-114°c), it does not disintegrate by heating , and the advantage of its shown persistence in acidic media and the lack of stability in alkaline media due to hydration ring lactone.[2]

Alcohol is Produced by micro-organisJms (bacteria - yeasts -fungi)by process of fermentation alcohol and lactic acid fermentation, which happened in aerobic and unaerobic conditions. *Saccharomyces cerevisiae* comes at the forefront of yeasts capable on alcohol fermentation [6][3], where sugar is converted into alcohol which is controlled by the The first stages of the alcohol fermentation process, while lactic acid fermentation occurs by is by lactic acid bacteria producing lactic acid , alcohol andJ ,carbon dioxide according to the following equations:

$$CH_3COCOO + H^+ \rightarrow CH_3CHO + CO_2$$

$$CH_3CHO + NADH_2 \rightarrow CH_3CHOH + NAD$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

2. The aim of search

Apple juice industry has been the remarkable progress recently in terms of both the amount of production or technologies in place, and the high nutritional value of apple juice and scientific advances in the field of human nutrition in the world are considered the main reason for the consumption of apple juice, as the fruit seasonal appearance is the main reason for the production the juice and that this trend of work permits and economically providing seasonal fruits as juice (easier to store) and to assess the validity of the juice for human consumption during storage periods The aim of search is:

1. Determination the chemical changes in the apple juice and its concentrates (total soluble solids concentration- total acidity - pH).

2. Determination patulin - alcohol - diacetyl in apple juice and its concentrates during storage periods.

3.Material and methods

Sodium hydroxide-potassium hydroxide – α- naphthol - phenol Vtalin- creatine- acetylated - sodiom carbonate - ethyle acetate - patulin - acetonitrile - distilled water - alcohol - apple juice.

4.Tools and devices

Isolation room JSCB-1200 SB (Korea), A high performance liquid chromatography /HPLC/, Spectroscopy, Incubator (UK) Genlabincubator, The pH meter, Balance of sensitive Mettlertoledo scale (Taiwan), Preheated with Magnetic Stirrer BIBBY B292 (UK), (France) , Autoclave, Water distillation device (GFL of the company - Germany), Refractometer, Tubes, Pipettes, Plastic lids, Flasks (250-500-1000) mL.

5.Research´s methods

- collecting juice samples:

we took in this work Twenty kg of apple juice concentrate (70%) from company of Natural Aljabal Juice from AL-suidaa Governorate with the following standards:

Name: Apple juice concentrate

Article: Aspetk / 100% Natural code

Material specifications: Appearance: Net Transparent - quality mass: 1.35

Chemical analysis: total soluble solids**:** 70% BIRX / 20° c

Sugars: glucose: 17.7 g / 100 g - fructose: 36.9 g / 100 g - sucrose: 9.6 g / g.

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strain, J.Bacteriol. (179):3362-3364.

[1] VANEGMOND, H., P., 1989- Current situation on regulations for mycotoxins. Overview oftolerances and status of standard methods of sampling and analysis. J. Food AdditContam.(6): 138–188.

[2] MERAI,A.,1991-Stadia and MikrobiologicznymiIChemiczymiZmianamPrzechowyWaniaKoncentratowSokuJablkawego. RozprawaDokotoskaWykonanw, Warszawa-Poland.

[3] MOSS, M.O., AND LONG, M.T.,2002- Fate of patulin in the presence of the yeast Saccharomyces cerevisiae. J. Food AdditContam. 19(4):387– 399.

Phenolic materials: 0.02-0.6% Phenols.
Nitrogenous materials: 0.2-1.5%.
Pectine: negative
Starh :negative
Total acidity: 12 g /L as malic acid
pH: 3.7/ 20 °c
Vitamin C: 100 mg / kg
Vitamin A: 100 mg / kg
Ash (mineral): 0.30-0.35%
Turbidity: 5 NTU / HACH method maximum /
We prarerd two concentarate (15% and 35%) by using distilled –sterlization water . these samples stoered at (4-20-30-40)ºc for 6 months, Chemical tests were assayed in the every month average two to every concentrate and all different temperatures and recored the average.
- **Chemical analysis:**
1-Determination of acidity:
According to the AOAC (2005) [1]
we took 10 g of juice samples and put in erlenmeyer flask with a capacity of it 250 ml and add boiling recently water to it even complete size and take 100 ml of diluted juice and added to 0.3 ml of phenol Vtalin to it and has been calibrated with a solution of sodium hydroxide (0.1N), expressed as % malic acid.

$$acidity\ \% = \frac{0.1 * \ size\ of\ NaOH\ ml * \ equlity\ weightof\ malic\ acid}{weight\ sample * 100} * 100$$

2- Determination of total soluble solids: According to [13] using Refractometer Kruss-Germany device.
3- Determine (pH): using pH meter [2]
4- Determination of diacetyl: According to [3]:
300 ml of apple juice concentration 12 % Brix was distillation.
Taking 25 ml of the distilled liquid was added to 10 ml of solution (α- naphthol + alcohol 95%) 5% and 4 ml of creatine + potassium hydroxide (40%),
The optical absorption measurement within a period 1 minute / 530 nm,
Measured the concentration of diacetyl using different standard solutions with concentrations of 0.1-50ppm (ppm).
We use (Methyl Red - Voges Proskaur) test which consists of two compounds (α- naphthol, NaOH 40%) to reveals diacetyl as well as the dye Blue instance this disclosure will be positive when you see the purple color when there is a partial oxidation of the composition of diacetyl. The figure shows (1) the curved Ayari for diacetyl indicating the relationship between the absorbance and the concentration of diacetyl

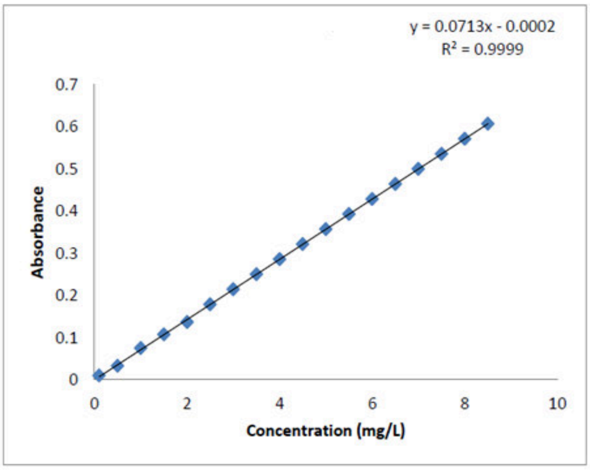

Figure (1) standard curved for Diacetyl

1 AOAC International.,2005- AOAC International, AOAC Official Method 995.10 Patulin in apple juice. In: W. Horwitz, Editor, Official Methods of Analysis of AOAC International (18th ed.), AOAC International, Gaithersburg, MD, USA (2005).
2 DRAZAZGA, B.,1984-Analizatechiczna w przetworstwieowocowI Warzyw Copyright by wydawnictwaSzkolne I pedagoiczne Warszawa- Poland.
3 GRZYBOWSKI, R., AND WAROWNA,K.,1991-Badanie Wplywu niektorych czynnikow na spoziom dwuacetylu w zageszczonych sokach cytrusowych,Przem.Spoz., 5/6 :143,Warszawa, Polanda.

5-Determine patulin: to according to the following steps[1]:
- Taking 25 g of the apple juice was added to 50 ml of ethyl acetate in a separating funnel.
- Mix the contents of funnel shaking for 5 minutes.
- Separation the two phases from each other and taking the organic phase which gathered only at the top of the separating funnel.
- Added 4 ml sodium carbonate 4% to the organic phase after separated it and mix them shaking again for 5 minutes.
- Separation the two phases from each other and taking the lower phase while keep the upper phase in the bottle.
- Organic phase steam by using evaporator under vacuum at temperature 40-50 °c until drying completely then the resulting melt in 1 ml of acetonitrile : water (10:90).
- Taking 10 microliter of previous sample and injection in high performance liquid chromatography device /HPLC/.
- Mobile phase is / acetonitrile : water/.
- Reading is taking on 254 nm .
- Figure (2) shows the amount of sattandrd patulin which measured on HPLC device:

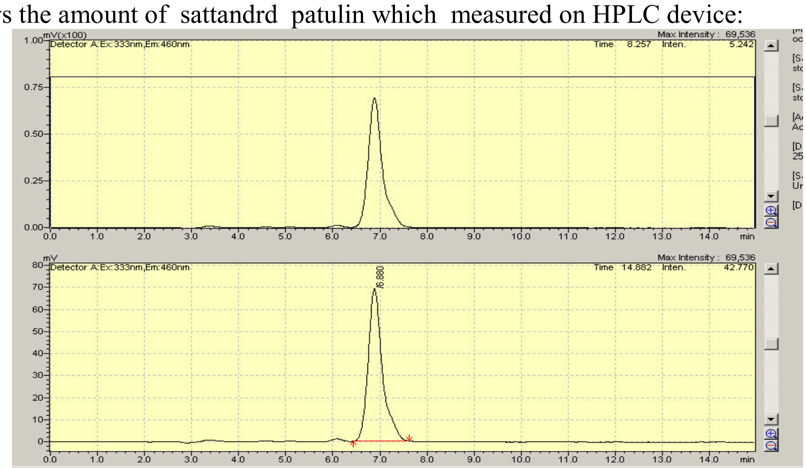

Figure (2) The amount of sattandrd patulin which measured on HPLC device

6-determining Alcohol: Use a rotary evaporator to set alcohol where 50 ml of apple juice was filled allocated evaporator flask in a rotary , temperature is determine less than 50 ° c, it heating until (78) °c and took all drops obtained when temperature is stabilited , measured it with pipette and expressed the result as a percentage volume (V / V) [11][2].

6. Statistical analysis

we use the General Linear Model to calculate the arithmetic mean and standard deviation also used the test (Turkey) to find significant differences between the averages using Minitab Statistical program at a confidence level 5% with 2 repeats for each experiment.

7. Results and discussion

7.1. Total soluble solids concentrate TSS

The tables 1,2,3 show morale changes in concentrate of samples apple juice 70% in all different temperature storage whereas The tables 2and 3 show clear changes in concentrate of samples apple juice 15% and 35% in the same storage conditions, the reduction in total soluble solids was more clear at temperatures 20 and 30 and exclusively at 20 that temperature is is agreeable for oraganisms to grow which consuming the composition of apple juice that form total soluble solids.

7.2. Acidity and pH

The tables 1,2,3 show low changes in ph valuas through storage periods espeacially at 20,30 this changes were moer clear for total acids in the first month at 20,30°c The reason for increase microbal activity and performs to unionize organic acids that enter to cell which reduce Acidity of plasma and ruin it, this high Acidity doesnot effect in pH.

[1] AOAC International.,2005- AOAC International, AOAC Official Method 995.10 Patulin in apple juice. In: W. Horwitz, Editor, Official Methods of Analysis of AOAC International (18th ed.), AOAC International, Gaithersburg, MD, USA (2005).

[2] GOSZCZ, H., 1988- Wybor I adaptac jametody ozaniapatuliny I Rozpozna wanie W zakresiejej Wystepo wania W przetworach Z Jablek Dokumentacja Pracuinstytutu Przemyslu fermentacyjnego,INA8, s. 15, Warszawa, Poland.

7.3. Patuline:

the amount of patuline that is determine with Liquid Chromatography (HPLC) was less than 50micro gram/kg in juice in all samples and under all condtation storage that is less than limit value for fumble device.

7.4. Diacytle:

The tables 1,2,3 show increase of amount in diacytle in samples of juice 15% (4.1±0.3) ppm in the end of second storage at temperature 20 ºc while the amount of apple juice 35%-70% was very little 1.9ppm at temperature 30 ºc for apple juice in the third month of storage and 1.69 ppm for apple juice 70% in the third month of storage at 20 ºc

7.5. Alcohol:

The tables 1,2,3 show the amonnt of alcohol relies to upper value (2.55 ± 0.1) % in apple juice 35% at 20 ºc in the sixth month of storage , the amount of alcohol in apple juice 20% was (1.25 ±0.4)% in the sixth month of storage and 0.52 % in apple juice 70% at 30ºc in the second month of storage.

Table (1) Chemical analysis in apple juice 15% during storage periods

Storage periods /Month/	Temperature /° c/	The chemical changes that associating to concentrates of apple juice			Microbial products		
		Total soluble solids % /TSS/	Ph	Acidity	Alcohol	Diacytle	Patuline *
Control	**Control**	**15.0±0.0**	**3.79±0.0**	**0.35 ± 0.0**	**0.00 ± 0.00**	**0.36 ± 0.0**	-
1	4	14.700±0.14	3.79± 0.01	0.46 ± 0.03	0.15 ± 0.07	1.25 ± 0.03	-
1	20	14.200±0.28	3.39± 0.03	0.51 ± 0.08	0.41 ± 0.14	3.18 ± 0.00	-
1	30	14.150±0.35	3.17±0.00	0.49 ± 0.03	0.44 ± 0.18	3.50 ± 0.14	-
1	40	13.900±0.14	3.28 ±0.01	0.45 ± 0.00	0.56 ± 0.07	2.14 ± 2.14	-
2	4	14.500±0.42	3.51± 0.04	0.58 ± 0.01	0.25 ± 0.22	1.63 ± 0.04	-
2	20	14.100±0.28	3.78 ±0.03	0.53 ± 0.01	0.46 ± 0.14	4.1 ± 0.3	-
2	30	13.800± 0.00	4.06 ± 0.43	0.51 ± 0.04	0.61 ± 0.00	3.40 ± 0.00	-
2	40	13.700± 0.42	4.43 ± 0.17	0.40 ± 0.01	0.66 ± 0.22	3.90 ± 0.14	-
3	4	14.500±0.42	3.51 ± 0.04	0.68 ± 0.00	0.36 ± 0.08	1.59 ± 0.04	-
3	20	14.100±0.28	3.78±0.0283	0.56 ± 0.04	0.46 ± 0.00	2.88 ± 0.06	-
3	30	13.80± 0.00	4.06 ±0.43	0.69 ± 0.01	0.61 ± 0.15	3.87 ± 0.00	-
3	40	13.70 ±0.42	4.43± 0.17	0.54 ± 0.06	0.66 ± 0.00	3.37 ± 0.06	-
4	4	13.60 ± 0.28	3.27 ± 0.03	0.58 ± 0.01	0.71 ± 0.15	1.45 ± 0.07	-
4	20	12.75 ± 1.06	3.48 ± 0.12	0.50± 0.01	1.15 ± 0.54	2.20 ± 0.14	-
4	30	13.00 ± 0.00	4.25 ± 0.08	0.765± .00	1.02 ± 0.00	1.95 ± 0.07	-
4	40	13.20 ± 0.14	4.31 ± 0.02	0.76 ± 0.02	0.92 ± 0.07	1.20 ± 0.28	-
5	4	13.20 ± 0.28	3.12 ± 0.03	0.53 ± 0.01	0.92 ± 0.14	0.99 ± 0.01	-
5	20	12.70 ± 0.99	3.445 ± 0.35	0.52 ± 0.01	1.17 ± 0.50	0.4 ± 0.57	-
5	30	13.05 ± 0.07	4.35 ± 0.078	0.79 ± 0.00	0.99 ± 0.03	0.35 ± 0.07	-
5	40	12.95 ± 0.07	4.41 ± 0.01	0.78± 0.02	1.04 ± 0.03	0.70 ± 0.28	-
6	4	13.10 ± 0.28	3.17 ± 0.03	0.52 ± 0.01	0.97 ± 0.14	0.76 ± 0.00	-
6	20	12.55 ± 0.78	3.65 ± 0.05	0.54 ± 0.01	1.25 ± 0.40	0.400 ± 0.14	-
6	30	12.95 ± 0.07	4.35 ± 0.06	0.815± .01	1.04 ± .03	0.15 ± 0.07	-
6	40	12.85 ± 0.07	4.41 ± 0.00	0.81 ± 0.01	1.09 ± .03	0.35± 0.07	-

*** Default patuline at detection border in HPLC**

Table (2) Chemical analysis in apple juice 35% during storage periods

Storage periods /Month/	Temperature /° c/	The chemical changes that associating to concentrates of apple juice			Microbial products		
		Total soluble solids % /TSS/	pH	Acidity	Alcohol	Diacytle	Patuline *
Control	**Control**	**35.00 ±0.0**	**3.83 ±0.0**	**0.64 ± 0.0**	**0.00 ± 0.00**	**0.54± 0.0**	**-**
1	4	34.50 ± 0.29	3.83 ± 0.04	0.83 ± 0.03	0.25 ± 0.15	0.89 ± 0.01	-
1	20	33.85 ± 0.35	3.04 ± 0.00	0.81 ± 0.014	0.59± 0.18	0.94 ± 0.03	-
1	30	34.00 ± 0.00	3.99 ± 0.01	0.81 ± 0.00	0.51 ± 0.00	0.91 ± 0.00	-
1	40	33.00 ± 0.00	3.98 ± 0.01	0.76 ± 0.04	1.02 ± 0.000	1.04 ± 0.03	-
2	4	33.40 ± 0.42	3.71 ± 0.04	0.81 ± 0.03	0.81 ± 0.22	1.14 ± 0.06	-
2	20	32.00 ± 1.41	3.93 ± 0.03	0.85 ± 0.00	1.53 ± 0.72	1.44 ± 0.01	-
2	30	33.50 ± 0.14	3.40 ± 0.29	0.74 ± 0.06	0.77 ± 0.07	1.56 ± 0.03	-
2	40	33.00 ± 1.41	3.70 ± 0.00	0.77 ± 0.01	1.02 ± 0.72	1.28 ± 0.04	-
3	4	33.20 ± 0.14	3.81 ± 0.06	0.78 ± 0.07	0.92 ± .07	1.19 ± .014	-
3	20	30.50 ± 0.28	3.95 ± 0.01	0.79 ± 0.04	2.30 ± .14	1.68 ± 0.04	-
3	30	33.00 ± 0.00	3.93 ± 0.0	0.70 ± 0.06	1.02 ± 0.00	1.90 ± 0.00	-
3	40	32.00 ± 1.41	3.99 ± 0.01	0.73 ± 0.00	1.53 ± 0.72	1.43 ± 0.14	-
4	4	31.95 ± 0.07	3.81 ± 0.06	0.78 ± 0.07	1.55 ± 0.03	1.22 ± 0.01	-
4	20	30.40 ± 0.14	3.99 ± 0.01	0.83 ± 0.04	2.35 ± 0.07	1.76 ± 0.08	-
4	30	32.40 ± 0.57	3.96 ± 0.00	0.73 ± 0.05	1.32 ± 0.29	1.40 ± 0.00	-
4	40	31.65 ± 0.07	3.99 ± 0.02	0.75 ± 0.01	1.71 ± 0.03	1.39 ± 0.01	-
5	4	31.30 ± 0.14	3.86 ± 0.06	0.80 ± 0.07	1.89 ± 0.07	1.04 ± 0.02	-
5	20	30.25 ± 0.21	4.04 ± 0.08	0.79 ± 0.03	2.42 ± 0.11	1.16 ± 0.09	-
5	30	32.00 ± 0.00	3.97 ± 0.01	0.79 ± 0.05	1.53 ± 0.00	0.80 ± 0.00	-
5	40	31.00 ± 0.00	3.95 ± 0.07	0.76 ± 0.01	2.04 ± 0.00	0.97 ± 0.11	-
6	4	31.20 ± 0.14	3.89 ± 0.01	0.80 ± 0.07	1.94 ± 0.07	0.89 ± 0.01	-
6	20	30.00 ± 0.00	4.06 ± 0.06	0.95 ± 0.01	2.55 ± .0.1	0.27 ± 0.38	-
6	30	3.95 ± 0.09	4.11 ± 0.02	0.93 ± 0.01	2.01 ± .04	0.30 ± 0.00	-
6	40	4.06 ± 0.09	3.99 ± 0.01	0.89 ± 0.01	1.99 ± 0.07	0.89 ± 0.01	-

*** Default patuline at detection border in HPLC**

Table (3) Chemical analysis in apple juice 70% during storage periods

Storage periods /Month/	Temperature /° c/	The chemical changes that associating to concentrates of apple juice			Microbial products		
		Total soluble solids % /TSS/	pH	Acidity	Alcohol	Diacytle	Patuline *
Control	**Control**	**70.00 ± 0.00**	**3.67 ± 0.00**	**1.68 ± 0.00**	**0.00 ± 0.00**	**1.06 ± 0.00**	**-**
1	4	69.60 ± 0.42	3.91 ± 0.02	2.80 ± 0.14	0.21 ± 0.23	1.23 ± 0.23	-
1	20	69.80± 0.00	3.89 ± 0.03	2.03 ± 0.04	0.10 ± 0.00	1.31 ± .16	-
1	30	38.4 ± 44.7	3.90 ± 0.05	2.05 ± 0.00	0.51 ± 0.72	1.26 ± 0.1	-
1	40	70.00 ± 0.00	3.78 ± 0.00	1.70 ± 0.28	0.00 ± 0.00	1.35 ± 0.01	-
2	4	69.40 ± 0.42	3.81± .014	2.00 ± 0.28	0.31 ± 0.22	1.66 ± 0.00	-
2	20	69.70 ± 0.14	3.83 ± 0.06	2.000 ± 0.00	0.15± 0.07	1.43 ± 0.06	-
2	30	68.98 ± 0.00	3.87± 0.03	2.30 ± 0.14	0.52 ± 0.00	1.37 ± 0.03	-
2	40	70.00 ± 0.00	3.94 ± 0.03	2.10 ± 0.14	0.00± .00	1.41 ± 0.07	-
3	4	69.40 ± 0.14	3.73 ± 0.00	1.50 ± 0.28	0.31 ± 0.07	1.18 ± 0.03	-
3	20	69.60 ± 0.57	3.86 ± 0.014	1.30 ± 0.28	0.21 ± 0.29	1.69 ± 0.00	-
3	30	69.60 ± 0.00	3.89 ± 0.08	1.27 ± 0.01	0.20 ± 0.00	1.59 ± 0.13	-
3	40	69.70 ± 0.28	4.05 ± 0.21	1.37 ± 0.03	0.15± 0.14	1.45 ± 0.03	-
4	4	69.20 ± 0.14	3.79 ± 0.00	1.48 ± 0.28	0.41 ± 0.07	1.25 ± 0.28	-
4	20	69.70 ± 0.42	3.91 ± 0.014	1.42 ± 0.28	0.15 ± 0.22	0.83 ± 0.00	-
4	30	69.85 ± 0.07	3.81 ± 0.04	3.74 ± 0.04	0.08 ± 0.03	0.57 ± 0.13	-
4	40	69.75 ± 0.07	3.90 ± 0.28	1.31 ± 0.03	0.13 ± 0.04	0.45 ± 0.03	-
5	4	69.40 ± 0.14	4.15 ± 0.07	1.50 ± 0.28	0.31 ± 0.08	1.26± 0.28	-
5	20	69.95 ± 0.07	3.89 ± 0.01	1.42 ± 0.28	0.03 ± 0.04	0.53 ± 0.00	-
5	30	70.00 ± 0.00	3.93 ± 0.1	3.74 ± 0.04	0.00 ± 0.00	0.44 ± 0.13	-
5	40	69.85 ± 0.07	4.0 ± 0.28	1.31 ± 0.03	0.08 ± 0.04	0.38 ± 0.04	-
6	4	69.80 ± 0.14	4.25 ± 0.07	1.50 ± 0.28	0.10 ± 0.07	1.04 ± 0.28	-
6	20	69.95 ± 0.07	3.93 ± 0.01	1.62 ± 0.26	0.03 ± 0.04	0.53 ± 0.00	-
6	30	69.95 ± 0.07	3.90 ± 0.1	3.79 ± 0.04	0.03 ± 0.04	0.29 ± 0.13	-
6	40	69.85 ± 0.07	3.85 ± 0.21	1.34 ± 0.01	0.08 ± 0.04	0.33 ± 0.02	-

*** Default patuline at detection border in HPLC**

8. Conclusions and recommendations:

- The apple juice is suitable environment for the growth of microorganisms at the right temperature , pH and provide nutrients and exposed to the chemical composition has changed as a result of storage and the growth of microorganisms.
- The amount of Diacytle, Patulin and Alcohol is a good indicator for contamination apple juice and the changing chemical composition and the extent of the validity of the juice for human consumption.
- The height of Diacytle and Alcohol at 20 ° C concentration in apple juice concentrate 15% is indicator to the decline in the juice specifications during storage and then unfit for consumption.
- The best temperature to save the apple juice is 4 ° c and concentration 70%, where the microbial activity is little.