Plant Archives Vol. 20, Supplement 1, 2020 pp. 4-8 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETECTION OF ACTIVE COMPOUNDS BY GC/MS IN EXTRACT OF RED CABBAGE *(BRASSICA OLERACEA)* AND THEIR EFFECT IN THE PRESERVATION OF RAW MILK

Adnan Wahhab Habeeb Al-Mudhafr
Department of Food Science, Faculty of Agriculture, University of Kufa, Iraq
Corresponding author: adnan.almudhafar@uokufa.edu.iq

Abstract

The Gas Chromatography/ Mass Spectrometer (GC/MS) method was used to detect the active ingredients in the water extract of the red cabbage and showed several peaks. It was found that most active groups of esters, phenols, Alkanes and carboxylic acids, including (1-octadecene, acid, 5-trime thylsilyl, oxy-methyl ester, 3-Carboxamide, Oxime, 2-trifluoromethylephenyl, Heptacosane, Triacontane and Hexacosane), all of which are antibacterial, different concentrations of water extract of red cabbage were added to raw milk and decreased microbiological numbers increased with concentration for different periods. 0.15% (0.009, 0019, 0.019, 0,039, 0.045, 0.51, 2.4, 13)×10^6 cfu/ mL compared to control treatment (0.03. 0.30, 2.3, 33, 44, 48, 58)×10^6 cfu/ mL For a period of seven days, storage at 4°C , but the molds and yeasts during the seventh day of storage amounted range reached At 0.15 as Log 0.0019, compared to the control treatment it was log 3.7348 . The ratio of acidity of raw milk reached 0.19 % and this indicates that the extract of red cabbage increases the shelf life of raw milk.
***Keywords*:** Active compounds, GC/MS, water extract, red cabbage, raw milk

Introduction

The Cabbage (*Brassica oleracea* var.) is highly nutritional, rich in minerals, vitamins, mono saccharide's and quantity of bioactive such polyphinols, anthocyanins, flavonols, glucosinolates (Volden *et al*., 2008). Red cabbage is called because of its red leaves. The color of the leaf is usually associated to the content of anthocyanin, which is a group of flavonoids that are mainly related to the age of the free and free radicals from their antioxidant properties. (Bacchetti, 2014), Anthocyanins are composed of anthocyanadins and sugars. The sugars act as a food reserve for after harvest, the high content of anthocyanins may lead to a longer storage period (Wiczkowski *et al*., 2012). And these benefits, it has a variety of phytochemicals and the cabbage is also a good source of sinigrin which is a Glucosinolates containing sulfur that are beneficial to human health. (Roy *et al.,* 2009). You can get some of these extracts of red cabbage that lead microbial growth delay and are antioxidants, which leads to improving the quality of food and nutritional value and most plant chemicals important are alkaloids and phenolic compounds, Tannins and flavonoids and that the presence of these materials make them important great food industry, (Edeoga *et al.,* 2005; Salama and Marraiki, 2010). Plant extracts pure and semi-purified and used as anti-microbial and this huge amount of plants located on the surface of the earth, but a small percentage known of them and some antibiotics for the bacteria to contain Tannins, phenolic and Flavonoids and alkaloids which possess antimicrobial properties and consumed by man as antibiotics and Flavors Analgesics and Food additives (Al-Manhel, 2015). The water extracts of the cabbage are used to conserve food because they contain natural substances that have a inhibitory effect on microorganisms causing diseases and food damage. The cabbage contains Thiosulfates, a natural antimicrobial agent, and the water and saline extracts of garlic and cabbage show the inhibition of microorganisms such as bacteria, fungi and yeast that are transmitted by food (Scollard *et al*., 2013; Mau and Hsieh, 2001). The aim of the research was to identify the active compounds in the red cabbage extract and its effect in prolonging the life of the raw milk.

Materials and Methods

Buying Red Cabbage from local markets in Iraq and access to raw milk from the fields of the College of Agriculture.

Methods:

Water Extractions:

Prepare the water extracts by taking 500 ml of distilled water with 25 g of each model and leaving for 30 minutes on a magnetic mixer, filtered with Buchner funnel through the Whatman No.1 filter Concentrate the concentrated filter with Rotary Vacuum Evaporator at 40°C The laboratory temperature is 23-25 ° C, then placed in containers and kept in the refrigerator until use (Al Manhel and Niamah, 2015) .

Detection of active compounds in extracts

Detected active compounds in plant extracts prepared using a gas chromatography connected VIR mass in GC/MS Laboratory in the laboratories of the Ministry of Science and Technology and carried out the injection process was automatic separation conditions in both MS / GC According to the company's instructions device manufacturer as in the table (1)

Table 1 : Automatic conditions for separation of GC / MS

Mass Spectrometer	Gas Chromatography
IonSource :EI	Column＃1Agilent 190915-433UI Hp-5ms
Source Temp. :230.00 ºC	Ultra Inert 60C-350C:30m×250m×0.25m
Quad Temp:150	Column Oven Temp. :60 ºC
Time :4.20 min	Injection Temp. :350.00 ºC
StopTime :650 min	Pressure :7.037 psi
ACQ Mode :Scan	Flow :0.9 ml /min
Thrreshold:0.150	AerageVelocity :34.772 cm/sec
Scan Speed :1.562(N=2)	Holdup Time 1.4379
Start m/z :30.00	Step point 60C
End m/z :700.00	Intial 3min
	Hold time 3min
	Post run50C
	Program
	＃1Rate 5C/min
	＃2Value 280C
	＃2Hold Time 5min

Microbiological examinations

1. Estimate total number of bacteria Total count:

- **Central Nutrient Nutrient Agar :** Use the supplied medium from Himedia company and attend according to the instructions of the processed company and use the mean to estimate the total number of bacteria Total Bacterial count.
- **Nutrient Broth :** Use the supplied medium from Himedia and attend as instructed by the processed company and use the medium to reactivate the isolates during studyy.
- **Method of counting :** The method of counting count was used in (APHA 1978).

2. Estimation of the total number of yeasts and Molds

- **Central Dextrose - Potato PD A :** Use the supplied medium from Himedia and attend as instructed by the processed company and use the medium to estimate the total number of yeast and yeast**.**
- **Total number of yeasts and molds Yeasts and Molds :** Followed the method contained in the (APHA, 1978)
- **Estimate the total number of colon coliforms :**
- **Central MacConkey Agar :** Use the supplied medium from Himedia and attend as instructed by the processed company. Use the medium to estimate the numbers of the colon**.**
- **Total colon bacteria :** The method used in (APHA ,1978) was followed except using the MacConkey Agar medium

Statistical analysis

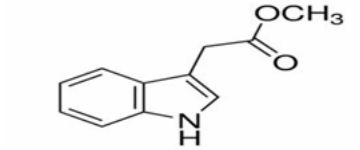
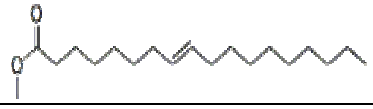
Complete Randomized Design (CRD) was used to analyze all the studied factors as statistically analyzed. These factors were tested using a least significant difference (L.S.D.) at a probability level of 0.05 using Genstat. Preserving the raw milk using red extract (GEN STAT, 2009).

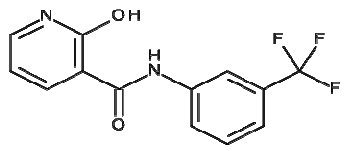
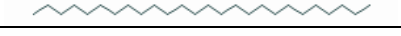
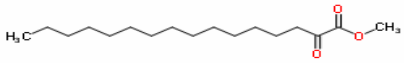
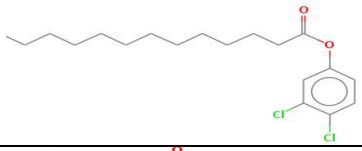
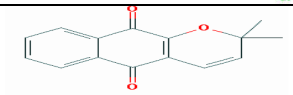
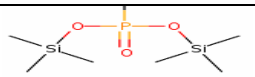
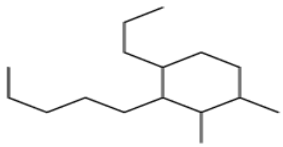
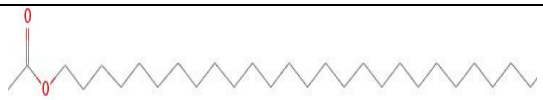
Results and Discussion

Active compounds in red cabbage extract

The results of the analysis and diagnosis of the active compounds in the GC/MS extract in Table (2) show the appearance of 15 active compounds main The results showed phenols, Most of these active groups were of esters, phenols, alkanes, alkyl halides, aldehydes, carboxylic acids, aromatic compounds, nitrite and amines. E-15-heptadecenal showed a molecular weight of 252.442 Da. and 1-octadecene in the top 1 concentration of 10.38% Microbial oxidation and antimicrobial In the pharmaceutical industry, 1H-Indole-3-acetic acid, 5-trime thylsilyl (oxy-methyl ester) appeared at top 2 and 0.88% concentration, acetic andol acid, which regulates plant growth and is present with growth-regulating hormones and catalase enzyme Octadecenoic acid methyl ester, which is the acidic acid, is noted for industrial and food applications. The Pyridine-3-Carboxamide, Oxime, 2-trifluoromethylephenyl is found in the top 4 concentration of 0.40%, an aromatic compound that coexists with antioxidant, anti-bacterial and inflammatory enzymes and enters the pharmaceutical and anti-wrinkle industries For oxidation, heptacosane, Triacontane and Hexacosane compounds appeared in the vertices (5, 6, 7) Acetate is an antibiotic and antimicrobial used in industrial applications. The top 8 contains Hexadecanoic acid, 2-oxo, methyl ester, which is used as a cosmetic preparation and is used as a food additive to improve myristic acid vinyl ester and myristic acid, 3,4-dichlorophenyl ester, esters of saturated fatty acid associated with antioxidant membranes used in the production of plasticizers and food packaging (9, 10), 23.35 %. concentration, and Xyloidone at the top 11, with a concentration of 29.58%, antibiotic and antibacterial. Pharmaceutical, and compound applications Phosphonic acid, methyl-bis (trim ethylsilyl) ester, an antiviral phosphoric acid used in the pharmaceutical industry. Its molecular weight is 240.38 Dalton. The compound that appeared at the top 13 is hexadecane-1,2-diol. The concentration of 4.74% is used in the pharmaceutical industry. The cyclohexane, 1,2-dimethyl-3 -pentyl4-propyl, an organic compound derived from the oxidation of selenium dioxide, is used in the pharmaceutical industry. The top 15 Heptacosyl acetate compound acetate is an anti-cause of pain molecular weight 438.77Da. These results were agreed with (Farag *et al*., 2010) when separating sulfur compounds and antioxidant compounds And anti-cancer of the Crusader family represented by the green faith and the red curse These results were agreed with (Cacciola *et al*., 2016) when segregating fatty acids and flavonoids from immunity and the results were not fully consistent with (Hou *et al*., 2016)

Table 2 : Active compounds in red cabbage extract, modified by GC/MS

Chemical composition	Name of compound	Code of Detention	No.
	E-15-Heptadecenal	**9-97-1000130**	1
	H-Indole-3-acetic acid,5-(trime thylsilyl) oxy-methyl ester1	**0-35-072101**	2
	Octadecenoic acid methyl ester E8	**7-50-026528**	3

	Pyridine-3-Carboxamide,Oxime, 2-trifluoromethylephen yl	7--**53-288246**	4
	Heptacosane	**7-49-000593**	5
	Triacontane	**6-68-000638**	6
	Hexacosane	**3-01-000630**	7
	Hexadecanoic acid ,2-oxo-,methyl ester	**1-30-055836**	8
	Myristic acid vinyl ester	**6-91-005809**	9
	Myristic acid ,3,4-dichlorophenyl ester	**93-1000357**	10
	Xyloidone	**4-92-015297**	11
	Phosphonic acid,methyl-bis(trim ethylsilyl)ester	**9-83-018279**	12
	Hexadecane,1-iodo	**4-77-000544**	13
	Cyclohexane,1,2-dimethyl-3-pentyl4-propyl	**4-17-062376**	14
	Heptacosyl acetate	**2-78-1000351**	15

Total count of microorganisms

Table (3) shows the total number of microorganisms of raw milk treated with cabbage extract. The results of the milk treated with the extract were 0.05% (0.03, 0.022, 0.049, 0.28, 2.3, 34, 52) $\times 10^6$ cfu/ml and for periods (5 hours)-1, 2, 3, 4, 5, 6, 7 days), while the number of microbiological milestones was 0.1% (0.012-0.021, 0.021, 0.045, 0.146, 0.32, 31, 44) $\times 10^6$ cfu / ml and the milk treated at 0.15% 0.009, 0.019, 0.019, 0.4, 13) $\times 10^6$ cfu/ml. The comparison treatment was the number of microorganisms (0.035, 0.3, 0.53, 2.3, 33, 44, 48, 58) $\times 10^6$ cfu/ml. Significant differences were observed $P < 0.05$ between the treatments. Because the microbial content in the process of conserving raw milk varies according to different concentrations. The ability of extracts to preserve due to their inhibited ability of microorganisms because they contain antioxidants such as phenols and each active material When using extract of leave plants that in the conservation of milk reduced the total number of microorganisms (Yemane *et al.*, 2016; Nyati, 2017).

Yeasts and molds Number

Shows (Table 4) The number of yeasts and molds in milk treatment models with different concentrations (0.05, 0.1, 0.15%) of the extract of red cabbage yeasts and molds did not appear in the milk laboratory models of these concentrations throughout the storage period while the preparation of yeasts and molds in comparison milk samples, The yeast and mold prepared by log (0.0019, 0.2521, 2.2932, 3.7348) when storage 7 days and through this we note the ability of the extract to inhibit the action of yeast and mold in the raw milk treated with different concentrations due to the high inhibitory efficiency towards yeast and mold as reported (Ay and Bostan, 2017).

Corrective acidity

It is noted from Table (5) that the average corrective acidity in the first few hours after adding the concentrations of the red milk extract to the cooled milk was within the acceptable limits (0.14-0.17)% while acidity developed after the third day of the control sample and reached 0.187%, and the acidity of the added milk sample was 0.05% of the red cabbage extract on the fourth day at 0.187. The acidity was also developed after the fifth day of the treatment at a concentration of 0.1% percentage of acidity at 0.186%. The acidity of the treatment was 0.185% the higher the concentration, the less acidity over time, and the significant differences (0.05) between the treatments. The high acidity is due to the high percentage of microorganisms that consume lactose sugar and its conversion to lactic acid, so the use of plant extracts reduces the proportion of microorganisms and thus decreases acidity (Yemane *et al.,* 2016).

Table 3 : Total count of microorganisms ($\times 10^6$ cfu/mL) of raw milk treated with the extract of cabbage For storage at 4 °C for different periods

Time Day	Concentrate %			
	0.05	**0.1**	**0.15**	**Control**
5 hour	0.03	0.012	0.009	0.035
1	0.022	0.021	0.019	0.3
2	0.032	0.021	0.019	0.53
3	0.049	0.045	0.039	2.3
4	0.28	0.146	0.045	33
5	2.3	0.32	0.051	44
6	34	31	2.4	48
7	52	44	13	58
	c	b	a	d

Table 4 : Log preparation of yeast and molds for milk treated with different concentrations of cabbage extract

Time (day)	Control	Conc. (%)		
		0.05	**0.10**	**0.15**
5 hour	2.8573	1.8213	0.213	0.0000
1	3.0606	1.9411	0.2227	0.0002
2	3.2174	2.0501	0.2302	0.0007
3	3.5705	2.135	0.2358	0.0012
4	3.638	2.2217	0.2411	0.0013
5	3.683	2.2482	0.2444	0.0015
6	3.7092	2.2782	0.247	0.0017
7	3.7348	2.2932	0.2521	0.0019

Table 5 : Corrective acidity of milk processed by the Red Cabbage extract

Conc.%				Time (day)
0.15	**0.10**	**0.05**	**Control**	
0.141	0.142	0.144	0.145	5h
0.147	0.148	0.153	0.155	**1**
0.149	0.155	0.162	0.175	**2**
0.155	0.162	0.175	0.187	**3**
0.163	0.175	0.187	0.21	**4**
0.175	0.186	0.191	0.25	**5**
0.185	0.191	0.21	0.25	**6**
0.191	0.21	0.25	0.27	**7**
a	b	**c**	d	

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