



The Effect of Active Compounds in Raisins on the Inhibitory Activity and Free Radical-Scavenging Capacity of Fortified Yogurt

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ABSTRACT

Background: Awareness has recently increased because of the reliance on plant extracts such as raisin extracts to enhance biological properties and produce functional products such as dairy products fortified with these extracts. **Methods:** In this study, the active compounds in aqueous and alcoholic extracts of black and yellow raisins were evaluated by GC-MS. The yogurt fortified with extracts was manufactured, and the diameter of the inhibition zone was estimated against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The free radical-scavenging capacity (DPPH%) was estimated at concentrations of 0, 5, 10, and 15 mg/ml. The letters also refer to antioxidants (A1) and (A2) in yogurt with alcoholic and aqueous extracts of black raisins and (A3) and (A4) in yogurt with alcoholic and aqueous extracts of yellow raisins. **Results:** The findings revealed the emergence of many active compounds, including alcohols, hydroxymethylfurfural, vitamins, alkanes, alkynes, phenolics, long-chain saturated fatty acids, organic acids, and other active compounds of different percentages. The highest inhibition zone of yogurt fortified with alcoholic extracts for black raisins was against *Bacillus cereus*, which reached 8, 20, and 23 mm, respectively, and increased in yogurt fortified with aqueous extracts for black raisins to 9, 21, and 24 mm, respectively. The free radical-scavenging capacities of the A1, A2, A3, and A4 samples were 38.17, 39.12, 39.46, and 39.89%, respectively, at a concentration of 15 mg/ml, whereas that of ascorbic acid was 85.90%. **Conclusion:** This study showed that consuming yogurt fortified with raisin extracts provides the body with functional benefits.

Introduction

One may keep fruit for a long period in a healthy way by drying it. Dehydrated fruits also have a longer shelf life than do their frozen or canned counterparts. According to (Maki and Yasin, 2023), dried fruits that are kept properly may be enjoyed for many years. Raisins, dates, and figs are among the most popular dried fruits; they are rich in nutrients and bioactive compounds that are good for people's health (Alasalvar *et al.*, 2023). Raisin extracts have a pleasant flavor, are

naturally sweet, and are rich in many nutrients. Cholesterol, saturated fat, and total fat are all present at minimal levels. Cardiovascular health is enhanced by the quantities of soluble and insoluble fiber that are supplied by these foods (Maki and Yasin, 2023). Yogurts may be made healthier by the addition of raisin extracts, which are high in dietary fiber and polyphenols and are thought to enhance physiological functioning (Ning *et al.*, 2021). According to study of Maki and Yasin,

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raisins contain many nutrients, including carbohydrates, nitrogenous compounds, ash, fibers, minerals (Fe, Cu, Mg, Ca), vitamins (B1, B2, B5), and antioxidants (Maki and Yasin, 2023). According to the another study, raisin extracts help the body absorb vitamins, minerals, and proteins correctly and have a calming effect on the neurological system, which makes it stronger (Alasalvar *et al.*, 2020). Raisins help the body eliminate toxins, avoid constipation, and are good for the heart and lungs (Sabra *et al.*, 2021). Raisin extracts contain phytochemicals, bioactive components, and high concentrations of polyphenol compounds, according to several studies (Ning *et al.*, 2021). According to Kaya *et al.* study, raisin extracts have long been considered popular healthy foods because of their good flavor, inherent resistance to deterioration, and ease of storage (Kaya *et al.*, 2024). The health and fitness of consumers are directly affected by the quality of raisins. In addition, patients, elderly individuals, and young people eat raisins because of their benefits (Sharma *et al.*, 2018).

Fermented milk products such as yogurt are highly desirable to consumers because of their health advantages, convenience, and ease of portability (Sobti *et al.*, 2021). According to Sobti *et al.* study, probiotics found in yogurt improve the qualities of naturally occurring microbes in the gut (Sobti *et al.*, 2021). For a long time, people have included dairy products in their diet, such as yogurt, fermented milk, and drinks, since they are considered good carriers for delivering probiotic bacteria to humans (Aspri *et al.*, 2020). Currently, there is increasing interest in foods that have health advantages beyond their nutritional content. There has been an increase in sales and an improvement in customer satisfaction with new kinds of yogurt that have distinct fragrances and flavors and may also have health benefits (Mohammadi-Gouraji *et al.*, 2019). The use of fruit and vegetable extracts for flavoring and fragrance enhancement has led to the creation of diverse yogurt that is well received, has better nutritional content, and promotes health (Ning *et al.*, 2021). There is a need to clarify whether the inclusion of fruit juices and their extracts may modify yogurt either favorably or adversely because yogurt has distinctive qualities that make it acceptable to customers (Dimitrellou

et al., 2020). According to Sarker *et al.* study, these goods have the potential to alter the acidification rate, fermentation duration, proliferation of starter bacteria, and other biological and major physicochemical properties of fermented dairy products (Sarker *et al.*, 2022). As far as the authors are concerned, there is presently a shortage of data concerning how raisin extracts influence the properties of yogurt. This study aimed to produce fortified yogurt using black and yellow raisin extracts as a natural food supplement to fill the information gap on how and to what extent the active compounds present in these extracts affect the inhibitory and antioxidant properties of yogurt.

Materials and Methods

Experimental design

Black and yellow raisins (**Figure 1**) were randomly obtained from local markets in Basrah/Iraq and extracted with water and alcohol. Raw cow milk was obtained from an agricultural research station/University of Basrah/Iraq.



Figure 1. Black (A) and yellow (B) raisins.

Extraction of black and yellow raisins

The fruits of black and yellow raisins were washed, cleaned and dried at 45 °C until the weight was fixed in an electric oven with ventilation racks (England) and ground with a Silver Crest (Germany). The methanol extract was prepared by mixing 1 g of raisin powder with 200 ml of methanol and distilled water (50:50 V/V) via a Shin Saeng magnetic mixer (Korea) for 1 hour, after which the mixture was left in opaque packaging for 24 hours at 4 °C and centrifuged at 2500 rpm for 10 min in Brookfield (USA). The aqueous extract was prepared by mixing 1 g of

raisin powder with 200 ml of distilled water at 60 °C for 30 min and then filtering it with Whatman No. 1 filter paper. The alcohol and aqueous extract were evaporated with a Heidolph vacuum evaporator (Germany), dehydrated with a Crest (Germany) and preserved at -20 °C pending tests (Arab *et al.*, 2024, Polat Kose *et al.*, 2020).

Estimation of active compounds in black and yellow raisin extracts by GC-MS

The active compounds in the milk samples were identified via a GC-MS device utilizing an HP-5 ms column and helium gas at a flow rate of 1 ml/sec. The injection temperature was 290 °C, and the GC oven program started at 40 °C. The temperature was increased to 300 °C over 20 minutes at a rate of 10 °C per minute. The separated peaks were matched with the spectral database from the NIST 2014 library (Yuan *et al.*, 2023).

Yogurt production

The process he described was used to make yogurt. A mixture of cow milk and other ingredients was pasteurized at 80 °C for 30 minutes, according to (Dimitrellou *et al.*, 2020). Water and 2% alcoholic extracts of black and yellow raisins were added after the mixture had cooled to 42 °C. The infected mixture was mixed with the starting culture YO-MIX Danisco (Denmark) 0.02% and incubated at 42 °C until the pH reached 4.6. Until the tests were carried out, the yogurt was kept in a refrigerator at 4 °C.

Determination of antimicrobial activity in yogurt fortified with black and yellow raisin extracts

Bacterial cultures: Pathological bacterial cultures were obtained from the Microbiology Laboratory of the Faculty of Agriculture, Basra, Iraq. Four different bacteria were examined for their inhibitory activity: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The isolates used for this investigation were activated for 24 hours at 37 °C in Hi-Media (India), a nutrient broth medium. It was compared with the McFarland solution with a turbidity of 0.5 and yielded an approximate number of bacterial cells of 1.5×10^8 CFU/ml after the turbidity was measured with a

spectrophotometer at 600 nm, after which it was incubated at 37 °C for 24 hours (AlKhafaji *et al.*, 2024, Polat Kose *et al.*, 2020). It should be noted that the negative effects of using raisin extracts on the positive bacteria in yogurt have not been tested.

Inhibition zone: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were spread on Mueller-Hinton agar medium using a sterile L-glass rod, and the antimicrobial activity was estimated in accordance with the method described by Khodja (Khodja *et al.*, 2021). Holes were then made with a sterile metal drill with a diameter of 6 mm, and 50 µl of yogurt was poured into the holes at concentrations of 0, 5, 10, and 15 mg/ml. For 24-48 hours, the plates were incubated at 37 °C, after which the inhibition zone diameter (mm) was recorded via a ruler based on the diameter of the clear zone surrounding the well.

Determination of free radical-scavenging capacity in yogurt fortified with black and yellow raisin extracts

The free radical 2,2 diphenyl-1-picrylhydrazyl (DPPH) was used as described previously (Tlay *et al.*, 2024) with a few adjustments as follows: the free radical scavenging capacity of Yogurt fermented with black and yellow raisin extracts was estimated. (A1) refers to antioxidants in yogurt with alcoholic extracts for black raisins %, (A2) refers to antioxidants in yogurt with aqueous extracts for black raisins %, (A3) refers to antioxidants in yogurt with alcoholic extracts for yellow raisins %, and (A4) refers to antioxidants in yogurt with aqueous extracts for yellow raisins %, at concentrations of 5, 10, and 15 mg/ml. The authors combined the samples (one gram of material in 30 ml of absolute ethanol) with the same volume of DPPH solution (60 µmol in absolute ethanol). A UV spectrophotometer (Biochromultrosopes, England) was used to measure the absorbance at 517 nm after the combination had incubated for 30 minutes in a dark environment. A reference solution of ascorbic acid (5 mg/ml) was also included.

Data analysis

To ascertain whether the differences were statistically significant in the mean coefficients at

the 0.05 level of probability, the data were analyzed via CRD, ANOVA, and LSD tests in Genstat software 12.1 (Schmuller, 2017).

Results

Active compounds in black and yellow raisin extracts by GC-MS

Many active compounds with biological benefits have been identified in aqueous and alcoholic extracts of black and yellow raisins, including fatty acids, alcohols, hydroxymethylfurfural, alkanes, alkynes, phenolic compounds, vitamins such as thymine, and other active compounds in all samples, where long-chain saturated fatty acids such as hexadecanoic acid, octadecenoic acid, oleic acid, lethane, and organic acids such as propenoic acid were observed. **Table 1** and **Figure 2** show the 48 peak-active compounds in the black raisin alcoholic extract, retention time (R.T. min.), and percentage of the upper region (area %). The highest percentage of these compounds was

methanamine and N-hydroxy-N-methyl, which reached an area of 9.42% after 4.362 min. The results in **Table 2** and **Figure 3** show the active compounds in the aqueous extract of black raisins. A total of 42 peaks, including hydroxymethylfurfural, pyran, 3-methoxy-2,2-dimethyloxirane, and oxetane, and other active compounds, such as oxirane, whose area% reached 10.66% and appeared 17.52 min later, were observed. **Table 3** and **Figure 4** show the active compounds in the alcoholic extract of yellow raisins, with 48 peaks of active compounds, including cyclopentanol acetate, which appeared at an area of 10.03% at 17.41 min, along with other active compounds. **Table 4** and **Figure 5** also show the appearance of 48 peaks of active compounds returned to the aqueous extract for yellow raisins, including 5-hydroxymethylfurfural, which appeared in an area of 17.79% at 14.73 min, along with other active compounds.

Table 1. Active compounds in alcoholic extract for black raisins.

Peak	Library/ID	R.T. min.	Area %
1	Methanamine, N-hydroxy-N-methyl	4.36	9.42
2	Butanal, 3-methyl-	4.74	5.14
3	Ethane, (methylthio)-	5.02	5.89
4	2-Imidazolidinethione	5.58	0.49
5	Dodecane, 1,1-dimethoxy-	5.96	0.39
6	2H-Imidazol-2-one, 1,3-dihydro-4-methyl-	6.31	0.53
7	Methane, nitro-	6.57	2.68
8	2-Furanmethanol	7.18	1.35
9	Acetamide, N,N'-carbonylbis-	7.72	4.43
10	3-Methylpentane-2,4-diyl dicarbamate (isomer 1)	8.03	0.96
11	1H-Imidazole, 4,5-dihydro-2-methyl-	8.54	0.15
12	2(3H)-Furanone, 5-methyl-	8.75	3.09
13	2-Butanethiol, 3-methyl-	9.29	0.30
14	Methyl 2-[ethoxy(methoxy)amino]-2-methylpropanoate	9.42	0.20
15	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	9.90	5.96
16	2-(tert-Butylamino)ethanol	10.25	2.38
17	Butanedioic acid, dimethyl ester	10.97	1.44
18	Ethanamine, N-ethyl-N-nitroso-	11.27	1.22
19	2,5-Piperazinedione, 3-methyl-	11.47	0.82
20	4,5-Diamino-2-hydroxypyrimidine	11.90	5.65
21	1,3,4-Thiadiazol-2-amine, N-2-propenyl-	12.12	0.56
22	Maltol	12.46	0.12
23	Pentanedioic acid, dimethyl ester	12.61	0.35
24	Ethanamine, N-ethyl-N-nitroso-	12.81	1.70
25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.96	4.10
26	Dimethylamine, N-(neopentyloxy)-	13.38	5.40
27	5-Hydroxymethylfurfural	14.26	2.87
28	2-Dimethylsilyloxypentane	14.51	6.23

29	2-Trimethylsilyloxy-1,3-butadiene	15.16	6.08
30	Heptanedioic acid, dimethyl ester	15.84	1.45
31	Methoxyacetic acid, 3-pentadecyl ester	16.53	0.90
32	D-Mannoundecane-1,2,3,4,5-pentaol	17.03	0.23
33	Lethane	17.31	4.29
34	Nonanedioic acid, dimethyl ester	18.43	0.23
35	Benzenemethanol, 3-fluoro-	18.81	0.09
36	Hexadecane	19.00	0.47
37	Nonanoic acid, methyl ester	19.31	0.52
38	2-Propenoic acid, pentadecyl ester	20.08	0.45
39	Methyl tetradecanoate	20.43	0.19
40	Octadecane	21.20	0.32
41	Dodecanedioic acid, dimethyl ester	21.77	0.24
42	Methyl 6,8-dodecadienyl ether	22.06	0.13
43	9-Hexadecenoic acid, methyl ester, (Z)-	22.32	0.57
44	Hexadecanoic acid, methyl ester	22.51	2.18
45	Cis-7-Hexadecenoic acid	22.68	0.23
46	N-Hexadecanoic acid	22.85	1.45
47	9-Octadecenoic acid, methyl ester, (E)-	24.19	2.90
48	9-Octadecenoic acid, (E)-	24.53	3.01

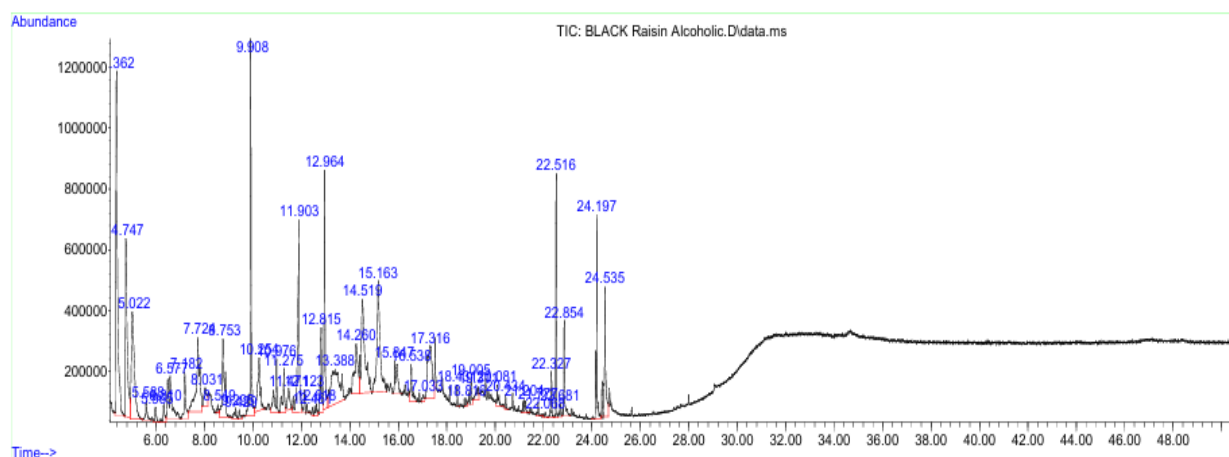


Figure 2. Active compounds in alcoholic extract for black raisins.

Table 2. Active compounds in aqueous extract for black raisins.

Peak	Library/ID	R.T. min.	Area %
1	3-Methoxy-2,2-dimethyloxirane	4.32	6.92
2	Oxetane, 2,2-dimethyl-	4.70	4.40
3	Methanamine, N-hydroxy-N-methyl-	4.99	4.88
4	2-Thiazolamine, 4,5-dihydro-	5.55	0.39
5	Methyl urinate	5.92	0.13
6	Cyclopentanone, 2-methyl-	6.27	0.55
7	Ethane, (methylthio)-	6.53	1.58
8	2-Furanmethanol	7.13	1.35
9	Dihydro-3-(2H)-thiophenone	7.75	3.78
10	Triethylphosphine	8.06	0.38
11	2-Cyclopenten-1-one, 2-hydroxy-	8.76	2.7
12	2-Pentanethiol	9.17	0.10
13	2-Furanmethanol, 5-methyl-	9.43	0.16
14	1,5-Anhydro-2-O-acetyl-3,4,6-tri-O-methyl-D-glucitol	9.75	0.26
15	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	9.90	6.18
16	Cycloheptanone	10.25	2.12
17	Dimethylamine, N-(neopentyloxy)-	10.62	0.32
18	Butanedioic acid, dimethyl ester	10.96	0.87
19	2-Thiazolamine, 4,5-dihydro-	11.27	1.41
20	5-Methylhexane-2,4-dione, enol	11.48	0.72
21	4,5-Diamino-6-hydroxypyrimidine	11.91	5.16
22	2-(2-Butoxyethoxy)acetic acid	12.13	0.18
23	Maltol	12.46	0.26
24	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.99	8.70
25	2-Butanone, 4-hydroxy-3-methyl-	13.66	4.73
26	5-Hydroxymethylfurfural	14.26	7.12
27	Ethanethioamide, N,N-dimethyl-	14.59	6.27
28	2,3,1-Benzodiazaborine, 1,2-dihydro-1-methyl-	15.23	6.43
29	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	16.01	1.27
30	Tetradecane	16.53	1.43
31	Oxirane, [(dodecyloxy)methyl]-	17.52	10.66
32	Hexadecane	19.00	0.33
33	3-Deoxy-d-mannonic lactone	19.31	0.91
34	Neopentyl alcohol, TBDMS derivative	21.24	0.26
35	12-Methyl-oxa-cyclododecan-2-one	21.77	0.15
36	Methyl hexadec-9-enoate	22.32	0.32
37	Hexadecanoic acid, methyl ester	22.51	1.24
38	cis-7-Hexadecenoic acid	22.68	0.12
39	n-Hexadecanoic acid	22.85	0.94
40	9-Octadecenoic acid, methyl ester, (E)-	24.19	1.66
41	Oleic Acid	24.53	2.19
42	1,2-Benzisothiazol-3-amine, TBDMS derivative	29.05	0.26

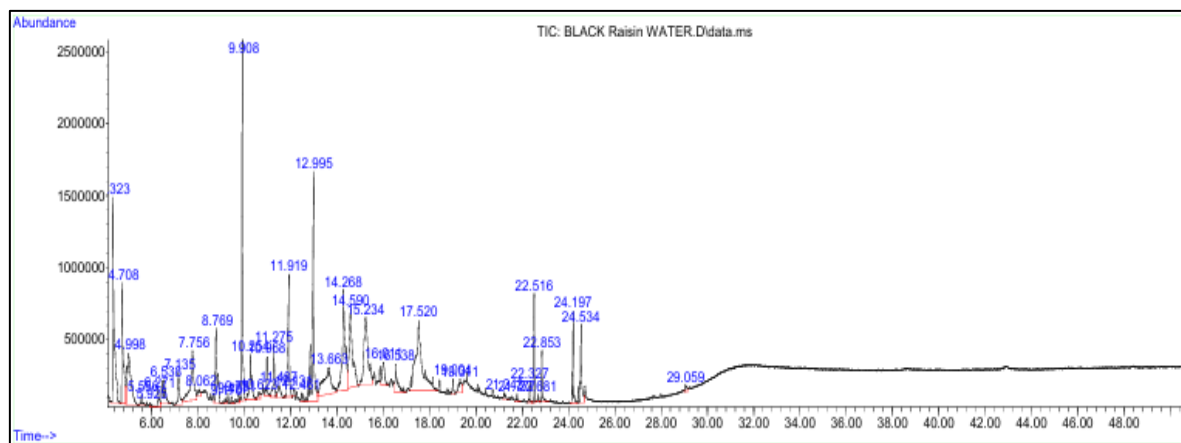


Figure 3. Active compounds in aqueous extract for black raisins

Table 3. Active compounds in alcoholic extract for yellow raisins.

Peak	Library/ID	R.T. min.	Area %
1	Methanamine, N-hydroxy-N-methyl-	4.33	7.83
2	Oxetane, 2,2-dimethyl-	4.73	4.26
3	Methanamine, N-hydroxy-N-methyl-	4.94	3.89
4	2-Imidazolidinethione	5.58	0.53
5	Diethyl 4,4'-methylenediallophanate	5.94	0.25
6	Cyclopentanone, 2-methyl-	6.29	0.26
7	2-Methyl-3-vinyl-oxirane	6.45	0.67
8	2-Furanmethanol	7.15	1.06
9	2-Thiazolamine, 4,5-dihydro-	7.73	3.95
10	Phosphine, diethyl-	8.03	2.54
11	Butanoic acid, 3-oxo-, propyl ester	8.48	0.32
12	Carbonic acid, isohexyl propyl ester	8.81	2.68
13	Boronic acid, ethyl-, bis(2-mercaptoethyl ester)	9.24	0.33
14	1,3-Oxathiolane	9.39	0.20
15	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	9.86	7.12
16	Oxirane, [(tetradecyloxy)methyl]-	10.23	2.71
17	Methyl 4-O-methyl-d-arabinopyranoside	10.63	0.13
18	Butanedioic acid, dimethyl ester	10.95	1.21
19	2-Thiazolamine, 4,5-dihydro-	11.25	1.44
20	1,3-Butadiene, 1-[(1-methylethyl)thio]-, (E)-	11.45	0.90
21	Thymine	11.91	7.49
22	1,3,4-Thiadiazol-2-amine, N-2-propenyl-	12.10	0.58
23	Maltol	12.45	0.20
24	Ethanamine, N-ethyl-N-nitroso-	12.80	1.80
25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.95	3.90
26	Dimethylamine, N-(neopentyloxy)-	13.24	2.80
27	1-Propanol, 3-methoxy-2-(methoxymethyl)-2-methyl-	13.66	0.36
28	5-Hydroxymethylfurfural	14.26	2.94
29	Ethanethioamide, N,N-dimethyl-	14.49	5.13
30	1-Propanol, 3-methoxy-2,2-bis(methoxymethyl)-	15.16	5.17
31	Heptanedioic acid, dimethyl ester	15.84	1.39
32	Butanoic acid, 3-methyl-, propyl ester	16.27	0.27
33	Tetradecane	16.53	1.29
34	Cyclopentanol, acetate	17.41	10.03
35	Nonanedioic acid, dimethyl ester	18.43	0.20
36	Tetracosane	18.99	0.88

37	Dodecanoic acid, 10-methyl-, methyl ester	19.31	0.67
38	.beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	19.47	0.42
39	2-Propenoic acid, pentadecyl ester	20.08	0.55
40	Methyl tetradecanoate	20.43	0.23
41	Dodecanedioic acid, dimethyl ester	21.77	0.29
42	9-Hexadecenoic acid, methyl ester, (Z)-	22.32	0.60
43	Hexadecanoic acid, methyl ester	22.51	2.28
44	Palmitoleic acid	22.68	0.25
45	n-Hexadecanoic acid	22.85	1.47
46	9-Octadecenoic acid, methyl ester, (E)-	24.19	3.03
47	Oleic Acid	24.53	2.90
48	Bis(2-ethylhexyl) phthalate	27.98	0.35

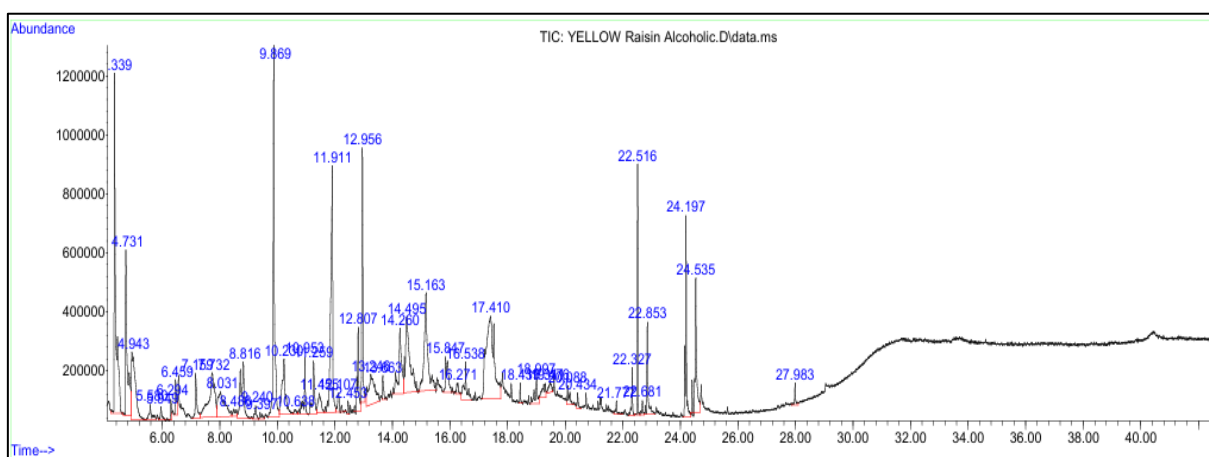


Figure 4. Active compounds in alcoholic extract for yellow raisins.

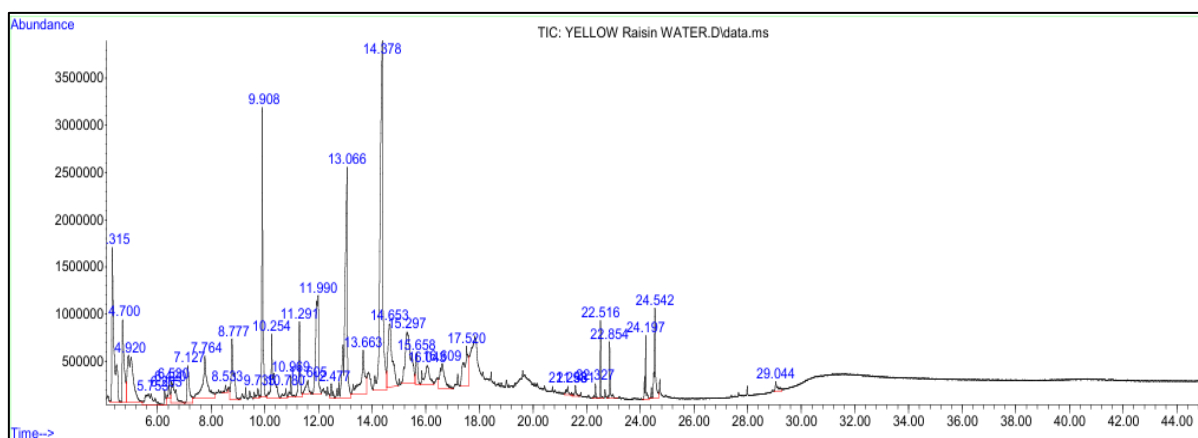


Figure 5. Active compounds in aqueous extract for yellow raisins

Table 4. Active compounds in aqueous extract for yellow raisins.

Peak	Library/ID	R.T. min.	Area %
1	Methanamine, N-hydroxy-N-methyl-	4.31	6.15
2	Butanal, 3-methyl-	4.70	2.98
3	Methanamine, N-hydroxy-N-methyl-	4.92	4.96
4	N'-(Diaminomethylidene)formohydrazide	5.75	1.52
5	Cyclopentanone, 3-methyl-	6.26	0.39
6	O-Butylisourea	6.42	0.39
7	Chloromethylmethyl sulfide	6.53	1.56
8	2-Furanmethanol	7.12	1.66
9	Butanamide, N-(aminocarbonyl)-3-methyl-	7.76	3.43
10	N-Acetyl-d-galactosamine	8.53	0.20
11	(R)-(1-Ethyl-2-pyrrolidinyl)methylamine	8.77	2.65
12	D-Gluconic acid, .gamma.-lactone	9.73	0.38
13	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	9.90	5.20
14	Cycloheptanone	10.25	2.72
15	But-2-enyloxy-dimethyl-silane	10.78	0.37
16	Butanedioic acid, dimethyl ester	10.96	0.38
17	2-Thiazolamine, 4,5-dihydro-	11.29	2.01
18	[1,3]Diazepan-2,4-dione	11.60	0.72
19	Thymine	11.99	5.09
20	Maltol	12.47	0.40
21	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	13.06	11.12
22	4(1H)-Pyrimidinone, 2,3-dihydro-5-methyl-2-thioxo-	13.66	3.10
23	5-Hydroxymethylfurfural	14.37	17.79
24	Ethanethioamide, N,N-dimethyl-	14.65	5.13
25	1-Propanol, 3-methoxy-2-(methoxymethyl)-2-methyl-	15.29	5.54
26	3-Piperidinol, 1-methyl-	15.65	1.06
27	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	16.04	1.72
28	Succinic acid, hept-2-yl 3-methylbut-2-yl ester	16.60	2.07
29	Dichloroacetic acid, dodecyl ester	17.52	3.02
30	Octanoic acid, 2-propenyl ester	21.29	0.32
31	1,3-Diazaspiro[4.5]decane-2,4-dione, 3-methyl-	21.58	0.30
32	6-Pentadecenoic acid, 13-methyl-, (6Z)-, O-methyl	22.32	0.22
33	Hexadecanoic acid, methyl ester	22.51	0.81
34	N-Hexadecanoic acid	22.85	0.86
35	9-Octadecenoic acid (Z)-, methyl ester	24.19	1.08
36	Oleic Acid	24.54	2.10
37	2-tert-Butylphenol, tert-butyldimethylsilyl ether	29.04	0.40

Antimicrobial activity in yogurt fortified with black and yellow raisin extracts

Table 5 show the inhibition zone diameter (mm) of yogurt made from cow milk fortified with alcoholic and aqueous extracts of black raisins. **Table 6** show the inhibition zone diameter (mm) of yogurt made from cow milk fortified with alcoholic and aqueous extracts of yellow raisins at concentrations of 0, 5, 10, and 15 mg/ml against four types of bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which are synergistic with yogurt and strawberry extracts.

There were no notable variations in the logarithm of the bacteria that were examined according to the statistical analysis, which had a significance threshold of $P < 0.05$. The tables show that the inhibition zone against all types of bacteria was tested, and the diameter of the inhibition zone increased with increasing concentration, as did the diameter of samples fortified with aqueous extracts of raisins compared with samples fortified with alcohol extracts, as did the diameter of all samples fortified with yellow raisins compared with samples fortified with black raisins. The antimicrobial activity of the

fermented yogurt samples was greater than that of the unfortified yogurt samples. The highest inhibition zone of yogurt fortified with alcoholic extracts of black raisins was against the Gram-positive bacterium *Bacillus cereus*. The inhibition zones measured 8, 20, and 23 mm at concentrations of 5, 10, and 15 mg/ml, respectively. For yogurt fortified with aqueous extracts, the inhibition zones against the same bacterium were 9, 21, and 24 mm, respectively. The table shows that the diameter of the inhibition zone in yogurt made from cow milk fortified with alcoholic and aqueous extracts for yellow raisins was greater than that in yogurt fortified with alcoholic and aqueous extracts for black raisins. The diameters of each of the yogurt samples enriched with *Bacillus cereus* were 9, 21, and 23 mm for alcoholic bacteria and 10, 22, and 24 mm for aqueous bacteria, respectively.

Free radical-scavenging capacity in yogurt fortified with black and yellow raisin extracts

Table 7 displays the DPPH free radical scavenging ability of cow milk yogurt enriched with alcoholic and aqueous extracts of yellow and black raisins. All samples were significantly different according to the statistical analysis $P < 0.05$. The results revealed synergistic effects between the raisin extracts and yogurt. (A1) refers to antioxidants in yogurt with alcoholic extracts for black raisins %, (A2) refers to antioxidants in yogurt with aqueous extracts for black raisins %, (A3) refers to antioxidants in yogurt with alcoholic extracts for yellow raisins %, and (A4) refers to antioxidants in yogurt with aqueous extracts for yellow raisins %, at concentrations of 5, 10, and 15 mg/ml. The samples were compared with ascorbic acid. The results revealed the free radical-scavenging capacity of the samples toward DPPH. An increase in these values was observed with increasing concentration. A slightly higher number of samples fortified with the aqueous extract than with the alcoholic extract were observed, as was the slight increase in all samples fortified with yellow raisin extracts compared with those fortified with black raisin extracts. The highest values for the A1, A2, A3, and A4 samples were 38.17, 39.12, 39.46, and 39.89%, respectively, at a concentration of 15

mg/ml. Compared with ascorbic acid, which exhibited a free radical-scavenging capacity of 85.90% at the same concentration, DPPH had a low capacity for all the samples.

Discussion

Active compounds in black and yellow raisin extracts by GC-MS

Dietary raisins, which include phenolic acids and flavonols, are the most consumed fruits and have strong antioxidant capabilities. According to studies (Jeszka-Skowron and Czarczyńska-Goślińska, 2020), these foods are regarded as beneficial to human health since they are rich in nutrients. One attribute typical of raisins is the presence of volatile compounds, which are influenced by the drying procedure and the type of grape used. The use of raisins as a component in processed foods may be beneficial since raisin volatiles include both free-form and glycosidically bound volatile chemicals (Wang *et al.*, 2017). Since ancient times, medicinal plants have been revered for their many pharmacological effects, which may be linked to the existence of secondary plant metabolites. Owing to their extensive range of chemical and biological actions, these compounds are important tools in the fight against and treatment of many human diseases (Sharma *et al.*, 2021). In addition to their antioxidant, anti-inflammatory, antibacterial, antiangiogenic, anticancer, and antiallergic actions, flavonoids and phenolic chemicals found in plants have a plethora of other biological effects. (Ralte *et al.*, 2022). These findings indicate that these bacteria contain biologically significant active compounds that act as natural antioxidants and antimicrobial agents. According to (Schuster *et al.*, 2017), both sundried and golden raisins are rich in phenolic acids and flavonol glycosides, making them a healthy addition to any diet. Triterpenes, fatty acids, flavonoids, amino acids, hydroxycinnamic acids, and 5-hydroxymethyl-2-furaldehyde are among the phytochemicals found in raisins. These

compounds have been found to suppress the growth of some infections (Rivero-Cruz *et al.*, 2008).

Table 5. The antimicrobial activity of yogurt made from cow's milk fortified with alcoholic and aqueous extracts for black raisins.

Tested strains	Control (mm)	Inhibition zone of yogurt with alcoholic extracts (mm)		
	0 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml
Alcoholic				
<i>Staphylococcus aureus</i>	6	6.00 ± 2.91	10.00 ± 2.91	13.00 ± 2.91
<i>Bacillus cereus</i>	6	8.00 ± 5.22	20.00 ± 5.22	23.00 ± 5.22
<i>Escherichia coli</i>	6	6.00 ± 3.12	9.00 ± 3.12	11.00 ± 3.12
<i>Pseudomonas auroginosa</i>	6	6.00 ± 3.08	9.00 ± 3.08	12.00 ± 3.08
Aqueous				
<i>Staphylococcus aureus</i>	6	6.00 ± 2.91	11.00 ± 2.91	14.00 ± 2.91
<i>Bacillus cereus</i>	6	9.00 ± 5.22	21.00 ± 5.22	24.00 ± 5.22
<i>Escherichia coli</i>	6	7.00 ± 3.12	10.00 ± 3.12	13.00 ± 3.12
<i>Pseudomonas auroginosa</i>	6	7.00 ± 3.08	10.00 ± 3.08	13.00 ± 3.08

Values are expressed as mean ± SD of three replicates.

Table 6. The antimicrobial activity of yogurt made from cow's milk fortified with alcoholic and aqueous extracts for yellow raisins.

Tested Strains	Control (mm)	Inhibition zone of yogurt with alcoholic extracts (mm)		
	0 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml
Alcoholic				
<i>Staphylococcus aureus</i>	6	7.00 ± 3.11	10.00 ± 3.11	14.00 ± 3.11
<i>Bacillus cereus</i>	6	9.00 ± 5.14	21.00 ± 5.14	23.00 ± 5.14
<i>Escherichia coli</i>	6	7.00 ± 2.72	10.00 ± 2.72	13.00 ± 2.72
<i>Pseudomonas auroginosa</i>	6	7.00 ± 4.17	10.00 ± 4.17	13.00 ± 4.17
Aqueous				
<i>Staphylococcus aureus</i>	6	7.00 ± 3.11	11.00 ± 3.11	16.00 ± 3.11
<i>Bacillus cereus</i>	6	10.00 ± 5.14	22.00 ± 5.14	24.00 ± 5.14
<i>Escherichia coli</i>	6	7.00 ± 2.72	11.00 ± 2.72	14.00 ± 2.72
<i>Pseudomonas auroginosa</i>	6	8.00 ± 4.17	11.00 ± 4.17	25.00 ± 4.17

Values are expressed as mean ± SD of three replicates.

Table 7. The antioxidant activity of yogurt made from cow's milk fortified with alcoholic and aqueous extracts for black and yellow raisins.

Samples	5 mg/ml	10 mg/ml	15 mg/ml
Ascorbic acid	75.78 ± 7.02 ^{ab}	78.95 ± 7.02 ^{ab}	85.90 ± 7.02 ^{aa}
A1	30.11 ± 7.02 ^{bc}	32.66 ± 7.02 ^{bb}	38.17 ± 7.02 ^{ba}
A2	30.25 ± 7.02 ^{bc}	33.56 ± 7.02 ^{bb}	39.12 ± 7.02 ^{ba}
A3	31.22 ± 7.02 ^{bc}	33.97 ± 7.02 ^{bb}	39.46 ± 7.02 ^{ba}
A4	31.38 ± 7.02 ^{bc}	34.15 ± 7.02 ^{bb}	39.89 ± 7.02 ^{ba}

The letters also refer to antioxidants (A1) and (A2) in yogurt with alcoholic and aqueous extracts of black raisins and (A3) and (A4) in yogurt with alcoholic and aqueous extracts of yellow raisins; Different letters in columns indicate the presence of significant

differences, and similar letters indicate no significant differences between the treatments at the level of probability ($P < 0.05$). Values are expressed as mean \pm SD of three replicates..

Antimicrobial activity in yogurt fortified with black and yellow raisin extracts

Raisin extracts contain many active compounds, such as hydroxymethylfurfural, oleic acid, linolenic acid, betulinic acid, furan methanol, and other active compounds (Rivero-Cruz *et al.*, 2008). Oleanolic acid has several pharmacological effects, including antitumor, hepatoprotective, cytotoxic, antidiabetogenic, and antimicrobial activities (Rivero-Cruz *et al.*, 2008). Neither extracts was very effective against gram-negative bacteria, despite the fact that it had some impact on gram-positive bacteria, this discrepancy may be caused by the structural difference between the cell walls of gram-positive and gram-negative bacteria (Abouzeed *et al.*, 2018). At very high concentrations, the alcoholic extract inhibited the growth of almost every bacterial strain tested (Soliman *et al.*, 2024). The synergistic antibacterial effects of raisins are enhanced by their high phenolic component percentages and the direct exposure of bacteria to these components (Soliman *et al.*, 2024), which might explain the antibacterial properties to their possible antibacterial properties, the bioactivity of grape polyphenolic compounds has attracted the attention of several researchers (Rivero-Cruz *et al.*, 2008).

Free radical-scavenging capacity in yogurt fortified with black and yellow raisin extracts

Essential nutrients, including vitamin C, E, β -carotene, anthocyanin, and flavonoids, are provided by the diet and work along with antioxidant enzymes to help the body's antioxidant defense system (Jacob, 2008). Fruits, vegetables, cereals, and other plant-based meals and drinks are rich in essential nutrients and antioxidant enzymes, which may have a role in preventing cancer, cardiovascular disease, and other disorders (Maki and Yasin, 2023, Noshad and Mehrnia, 2025). Fruit juices added to yogurt, including berries and grapes, increase its free radical-scavenging potential. Ruminant milk often contains high concentrations of phenolic chemicals, which are likely produced from the animal's diet or the byproducts of amino acid breakdown (Dimitrellou

et al., 2020). Microbes break down polyphenolic chemicals into smaller units in extracts that promote health, such as pomegranate or berry peel extracts. Given that bacterial culture may change the anthocyanin content while preserving it, it is likely linked to the starting culture producing antimicrobial chemicals (Jany *et al.*, 2024).

The research is well-organized, error-free, and features high-quality and reliable sources, clear wording, and sound data analysis. It should be noted that the research used aqueous and alcoholic extracts of yellow and black currants.

Conclusions

One promising area for future growth in the dairy sector is functional dairy products. The nutritional value of functional dairy products is well documented, and these products can be further enhanced by adding biologically active substances that promote human health. One significant technique to make functional dairy products is to add functional plant components such as dried and fresh fruits and grains, as well as their juices and extracts, such as black and yellow raisins, to the dairy. Polysaccharides, oligosaccharides, saponins, phytosterols, vitamins, and countless other physiologically active substances are added to dairy products. In addition to improved antibacterial and antioxidant capabilities, these fortified dairy products offer a variety of benefits, including hypoglycemic, lipid-lowering, immune-enhancing, anti-inflammatory, and other benefits.

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Authors' contributions

Al Musa RS. participated in the initial design, writing, editing, final revision of the manuscript and statistical analysis and Ethafa ES in laboratory methodology, sample preparation, statistical analysis. The authors have read and approved the final version of the manuscript for publication.

Conflict of interests

The authors have no conflict of interest.

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