Final Report for the California Raisin Marketing Board.

Title: Phenolic Content, Antioxidant Activity and Antimicrobial Properties of Raisins in Food Systems

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Summary

Phenolic compounds are becoming of interest as researchers are discovering functional activities as drugs, colorants, flavors, and antioxidants. Some phenolics share some biological and chemical properties that might be effective inhibitors of chemical mutagens and/or carcinogenesis. Previous studies have shown that naturally occurring hydroxycinnamate derivatives have antimicrobial activity. Phenolic content is also closely associated with the sensory and nutritional quality of fresh and processed plant foods.

Experiments were conducted to determine the type and amount of phenolics present in raisins and test their extracts to identify possible antioxidant and antimicrobial activity.

Raisins seem to have a considerable content of phenolic compounds. Golden raisins showed the highest concentrations, which can be due to the prevention of browning by SO2 application. Anthocyanins were not detected in any of the raisins analyzed. Golden raisins also showed three times higher antioxidant activity than Thompson-seedless and Zante raisins. Tentative identification of the phenolic profile by HPLC suggests the presence of quinic and gallic acid, chlorogenic and caffeic acids, catechin, epicatechin, and others yet to be identified. Water and methanol extracts from raisins greatly inhibited the growth of L. monocytogenes and E. coli 0157:H7 in the ranges of 70-95% and 50-70% respectively. This results where comparative to a control and 2% chlorogenic acid solution.

Application of raisin extracts to reduce browning in fresh-cut apples was studied. The antioxidant effect was more evident in Golden raisins compared to Thompson seedless. When raisin extracts were applied as antimicrobial pathogenic agents in fresh-cut fruits and vegetables, we observed that the protective effect depended on the type of produce utilized. In general there was a larger reduction in microbial population when using Golden raisin extracts compared to the control.

Our results suggest that raisins have antioxidant and antimicrobial properties that are related to their phenolic content. Even though much of the phenolics is lost because of browning reactions, the drying process when making raisins, concentrates the remaining amounts and make them significant on per weight basis.

These results open the possibility of utilizing raisins and raisin products to reach new markets where antimicrobial and antioxidant properties are needed. Additionally, our results indicate the possibility of creating new products from raisins, such as raisin powder, to concentrate phenolics even more and make them more functional.

TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN RAISINS.

Plants produce a large, diverse array of organic compounds that do not appear to have any direct function in growth and development. These compounds were becoming of more interest in as researchers were discovering functional activities as drugs, poisons, flavors, and industrial materials. Phenolic compounds are a very important group that comprises this type of compounds called "natural products". Phenolics are bioactive substances occurring widely in food plants. The phenolic content is closely associated with the sensory and nutritional quality of fresh and processed plant foods. Browning of certain food products results from both enzymatic (PPO) and non-enzymatic oxidation of phenolic compounds. Browning usually impairs the sensory properties of products because of the associated changes in color, flavor and softening. However, browning is sometimes desirable as it can improve the sensory properties of products like dark raisins and fermented tea leaves.

It has been known for decades that some substances present in commonly consumed foods reduce the incidence of chemically induced carcinogenesis in laboratory rodents. Certain plant phenols can be effective inhibitors of chemical mutagens, *in vitro*, and/or carcinogenesis *in vivo*. Many phenolic compounds share several common biological and chemical properties: (a) antioxidant activity, (b) the ability to scavenge active oxygen species, (c) the ability to scavenge electrophiles, (d) the ability to inhibit nitrosation, (e) the ability to chelate metals, (f) the potential for autooxidation, producing hydrogen peroxide in the presence of certain metals, and (g) the capability to modulate certain cellular enzyme activities. Some phenolics also share some biological and chemical properties with vitamins C and E, and many have been found to inhibit, or are very likely to participate in the inhibition of the steps of tumor development in experimental animals and probably in humans. Hence the importance of determining the amount and type of phenolic compounds in the food source.

For this first summary, we present the evaluation of some parameters that contribute to Thompson Seedless raisins quality and stability.

- pH 3.80
- Total Soluble Solids .....79.80 %
- Water Activity 63.70
- Moisture Content 28.95*
  (* conventional oven at 105°C x 24 hrs until constant weight)
- Moisture Content.....16.46 +/- 0.30**
  (** vacuum oven at 70°C x 24 hrs until constant weight)

Total phenolic content was evaluated by the Folin-Ciocalteau phenol reagent assay (Hyodo et al., 1978; Talcott and Howard, 1999), performing the proper adjustments to apply it to Raisins. The total amount of phenolics in both raisins and blueberries seems to be similar. However, the differences must be given by the type and specific amounts of the individual components.

PRODUCT	Phenolic Content (mg/100g)			Avg Phen. Cont	Stadev
	R1	R2	R3	mg/100g	
Raisins	536.70	529.89	476.58	514.39	32.921
Blueberries, TX	647.72	523.49	474.44	548.55	89.317

Determination of total anthocyanin content was performed spectrophotometrically according to Fuleki and Francis (1968), again with the proper corrections to adjust the method to raisins. Even though some traces of anthocyanins were detected, we consider they don't exist in Thompson Seedless raisin's tissue. The traces detected could be due to some yellow/brown pigments from phenolic oxidation, maillard reaction products or both, that were extracted by solvent used in the assay.

PRODUCT	Anthocyanin Content (mg/100g)				*Avg Anth. Cont*	*Stadev*
	R1	R2	R3		mg/100g	
Raisins	3.14	1.08	1.24		1.82	1.146
Blueberries, TX	85.44	96.68	85.55		89.22	6.458

In the evaluation of antioxidant activity of our raisin's extracts, the extracts were allowed to react with a stable radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) in a methanol solution (Brand-Williams et al., 1995). In it's radical form, DPPH• absorbs at 515 ηm, but upon reduction by an antioxidant (AH) or a radical species (R•), the absorption disappears

$$DPPH\bullet + AH \text{ ---------- } DPPH\text{-}H + A\bullet$$
$$DPPH\bullet + R\bullet \text{ ---------- } DPPH\text{-}R$$

The use of DPPH• provides an easy and rapid way to evaluate the antiradical activities of antioxidants. We prepared two standard curves, one with Ascorbic Acid (vitamin C) and one with Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a very powerful antioxidant), to use as a reference with our extracts. We also evaluated the antioxidant activity of blueberries to establish a comparison with our raisin's extracts. When compared as Vitamin C, raisins show a 33.7% of the activity of this antioxidant in blueberries. And when compared as Trolox, raisins only have 37.28% of that in blueberries.

PRODUCT	Ascorbic Ac. Equivalent.	Trolox Equivalent
	(μg/g of raisins)	(μg/g of raisins)
Raisins	446.660	723.311
Blueberries, TX	1323.00	1940.00

ANALYSIS OF RAISIN EXTRACTS FOR PHENOLIC TYPE AND AMOUNT

We conducted experiments to determine the type and amount of phenolics present in raisins from Thompson seedless grapes and Zante grapes. Composition analysis of the raisins extracts was performed by HPLC and the use of standards.

Type and amount of phenolic compounds

Phenolic content in raisins was characterized and quantified by methanolic extracts. Four different types of raisins were analyzed; regular raisins from Thompson seedless grapes (Thompson), raisins exposed to SO_2 while drying (Golden), raisins dipped in sugar solution previous to drying process (Dipped), and raisins from Zante grapes (Zante). An amount of 5 g was homogenized with 15 ml of methanol. After filtering and centrifuging, an aliquot was taken and concentrated with a rotavapor eliminating all the solvent. The concentrate was dissolved with water and passed through an Oasis HLB extraction cartridges to eliminate sugars and all water-soluble substances. The phenolics were washed out from the cartridge with methanol and concentrated again untill completely dried. The extract was dissolved in 1 ml methanol, filtered through a nylon 0.45 μm filter and analyzed with and HPLC system. A Spectra Physics model P2000 HPLC system was used fitted with a 3.9 × 150 mm C_{18}-silica gel column (Nova-Pak, Waters). Phenolic content was detected at 280 nm with a Perkin-Elmer LC295 UV-Vis detector (Norwalk, CT). The following gradient was used for the separation:

Time (min)	%A	%B	Flow rate (ml)
0	100	0	1
40	80	20	1
60	0	100	1
64	0	100	1
66	100	0	1

Solvent A was Water:Formic acid (19:1) solution, and solvent B was Acetonitrile:Formic acid (19:1). All solvents were HPLC grade. Phenolics were tentatively identified and quantified using standards from Sigma Chemical Co.

Results

Type and amount of phenolic compounds

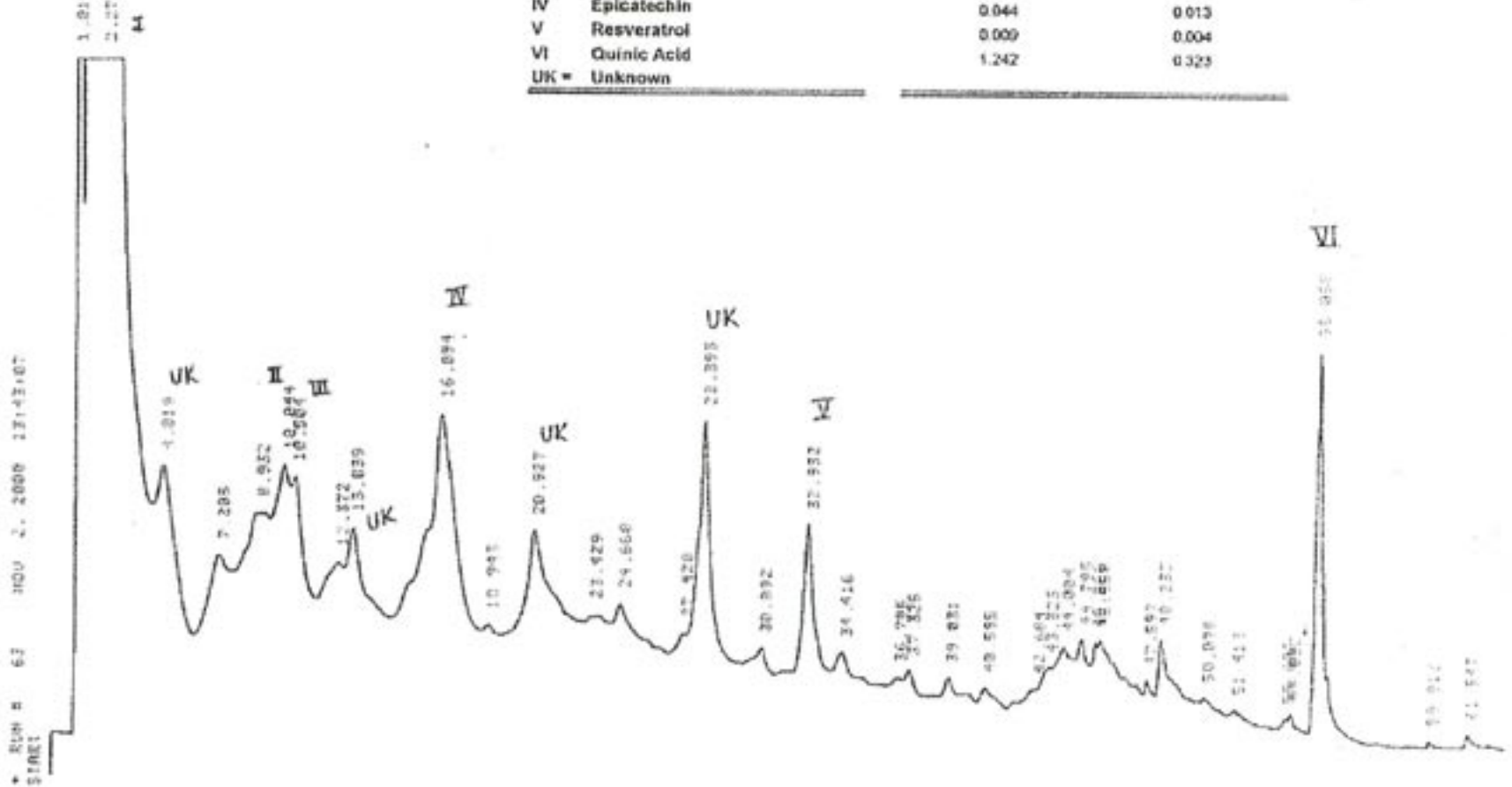
Grapes have been found to posses many phenolics with characteristic, functional properties. However, much of the amount of phenolics in grapes is lost because of the browning reactions occurring during the drying process. Many phenolics go through oxidation reactions carried out by Polyphenoloxidase (PPO). The enzymatic browning reaction in fruits often has been considered to be a linear function of the phenolic content and polyphenoloxidase activity. The final products of that oxidation process are the polymerized brown pigments that give raisins their color. Nonetheless, the weight loss during the drying process may also concentrate the small amounts left and make them significant on per weight basis.

Previous studies have shown that naturally occurring hydroxycinnamate derivatives have antimicrobial activity. However, there always are questions about their effectiveness. Some phenolics that work inhibiting some microorganisms may not be effective showing activity against other microorganisms. The chromatograms for the different types of raisins are shown. There are still some peaks that need to be identified. The peak appearing at 27-28 min seems to be a major component for all four types of raisins, and it would be very interesting to characterize it. Some other peaks showing early in the run seem of importance. Of the ones that were tentatively identified, quinic acid is the one that shows the highest concentration (especially in Golden raisins and Thompson seedless raisins at 134mg/100g and 124mg/100g, respectively) and appears in all types of raisins (e.g., Dipped, 2.3mg/100g; Zante, 0.9mg/100g). Other phenolic compounds present were Gallic acid in Zante raisins at 79mg/100g and Kaempherol in Dipped raisins at 46.3mg/100g. The use of sulfites has been widely used to avoid oxidation reactions and subsequent browning of food. Small concentrations of caffeic and chlorogenic acid were detected. These two hydroxycinnamic acids are found in many fruits and vegetables in their free state and in a variety of derivatives. Chlorogenic acid is present in coffee beans (4%), prunes (0.9%), blueberries (0.2%), apples (0.1%), pears (0.2%), and grapes (0.2%). Chlorogenic and caffeic acid have been found to inhibit tumorigenicity in various animal models, among other functional properties. Other phenolics identified were catechin, epicatechin. The antioxidant activity of these flavan monomers, which have a saturated ring lacking carbonyl group in the center of the molecule, is poor. However, the activity of the oligomers of these flavans is intensified by extended oligomerization and reinforced by the presence of galloyl group, like epicatechin gallate. Also a flavonol, like quercetin, having the same number of hydroxyl groups as these flavans and also an enol group conjugated with carbonyl group, exhibits potent activity. Grapes are good sources of quercetin, but seems to be lost during the browning reactions.

The presence of different amounts of specific phenolics in each type of raisin may have an effect on their bioactive and functional properties. In the following studies we will characterize the antioxidant and antimicrobial properties of raisins extracts. However, it is proposed that in future studies specific active fractions should be identified

for an array of possible uses, going from fraction separations to improving actual process operations of raisin and raisin product making, so as to maintain appropriate amounts of the valuable active compounds.

Phenolic Comp. In Thompson raisins		Concentration (mg/g of raisins)	
		Avg	Stdev
I	Gallic acid	0.005	0.000
II	Caffeic acid	0.004	0.000
III	Chlorogenic Acid	0.009	0.004
IV	Epicatechin	0.044	0.013
V	Resveratrol	0.009	0.004
VI	Quinic Acid	1.242	0.323
UK =	Unknown		



Phenolic Comp. In Dipped raisins		Concentration (mg/g of raisins)	
		Avg	Stdev
II	Caffeic acid	0.015	0.004
III	Chlorogenic Acid	0.012	0.001
IV	Epicatechin	0.010	0.001
V	Resveratrol	0.016	0.000
VI	Quinic Acid	0.023	0.000
VII	Catechin	0.008	0.005
X	Kaempherol	0.403	0.000
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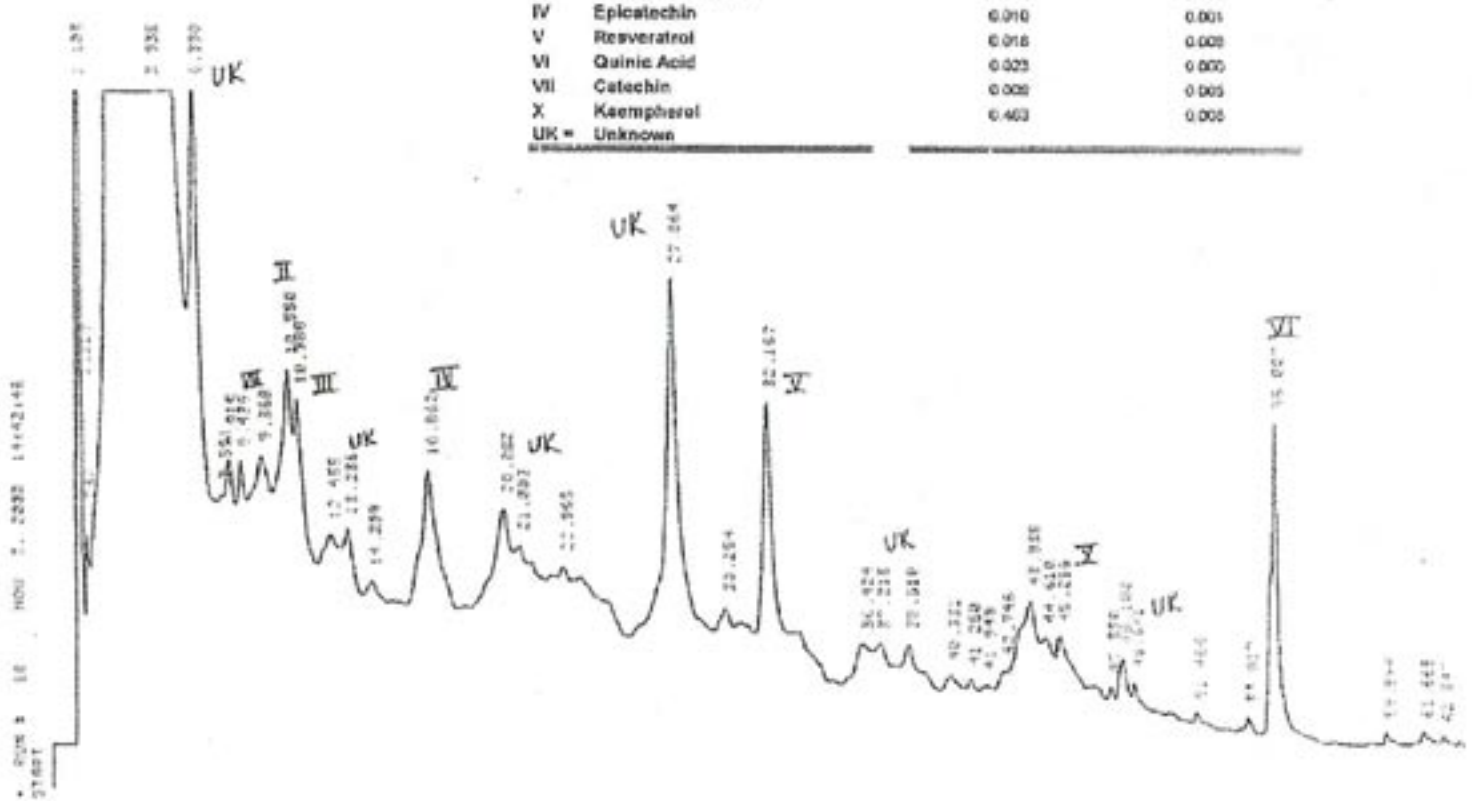

Phenolic Comp. In Golden raisins		Concentration (mg/g of raisins) Avg	Std. Dev.
II	Caffeic acid	0.018	0.005
IV	Epicatechin	0.004	0.000
V	Resveratrol	0.006	0.001
VI	Quinic Acid	1.341	0.002
VII	Catechin	0.094	0.002
VIII	Epicatechin Gallate	0.008	0.003
IX	Quercetin	0.003	0.000
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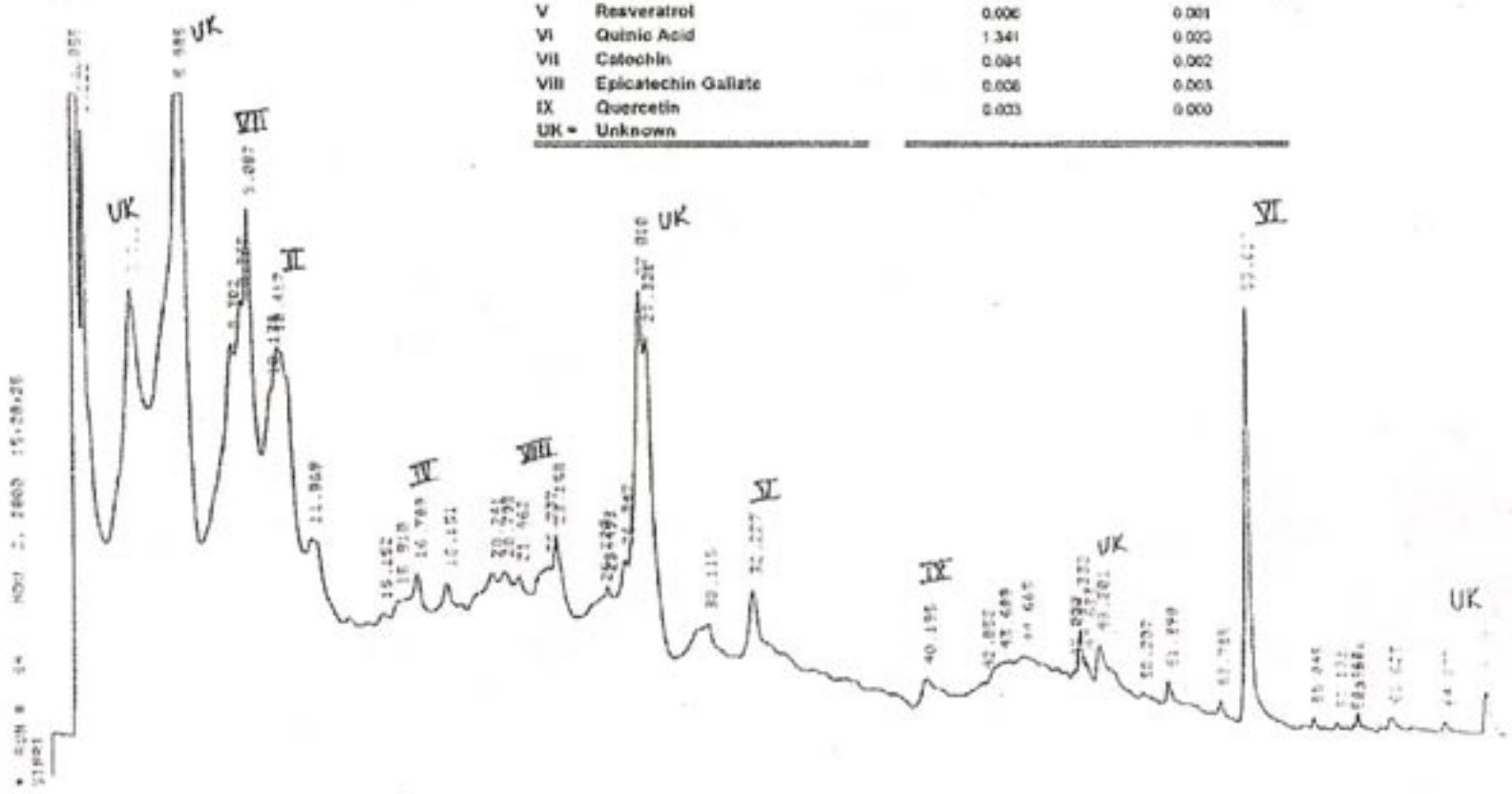

Phenolic Comp. In Zante raisins		Concentration (mg/g of raisins)	
		Avg	Stdev
I	Gallic acid	0.708	0.109
II	Caffeic acid	0.018	0.000
IV	Epicatechin	0.041	0.011
V	Resveratrol	0.022	0.006
VI	Quinic Acid	0.009	0.001
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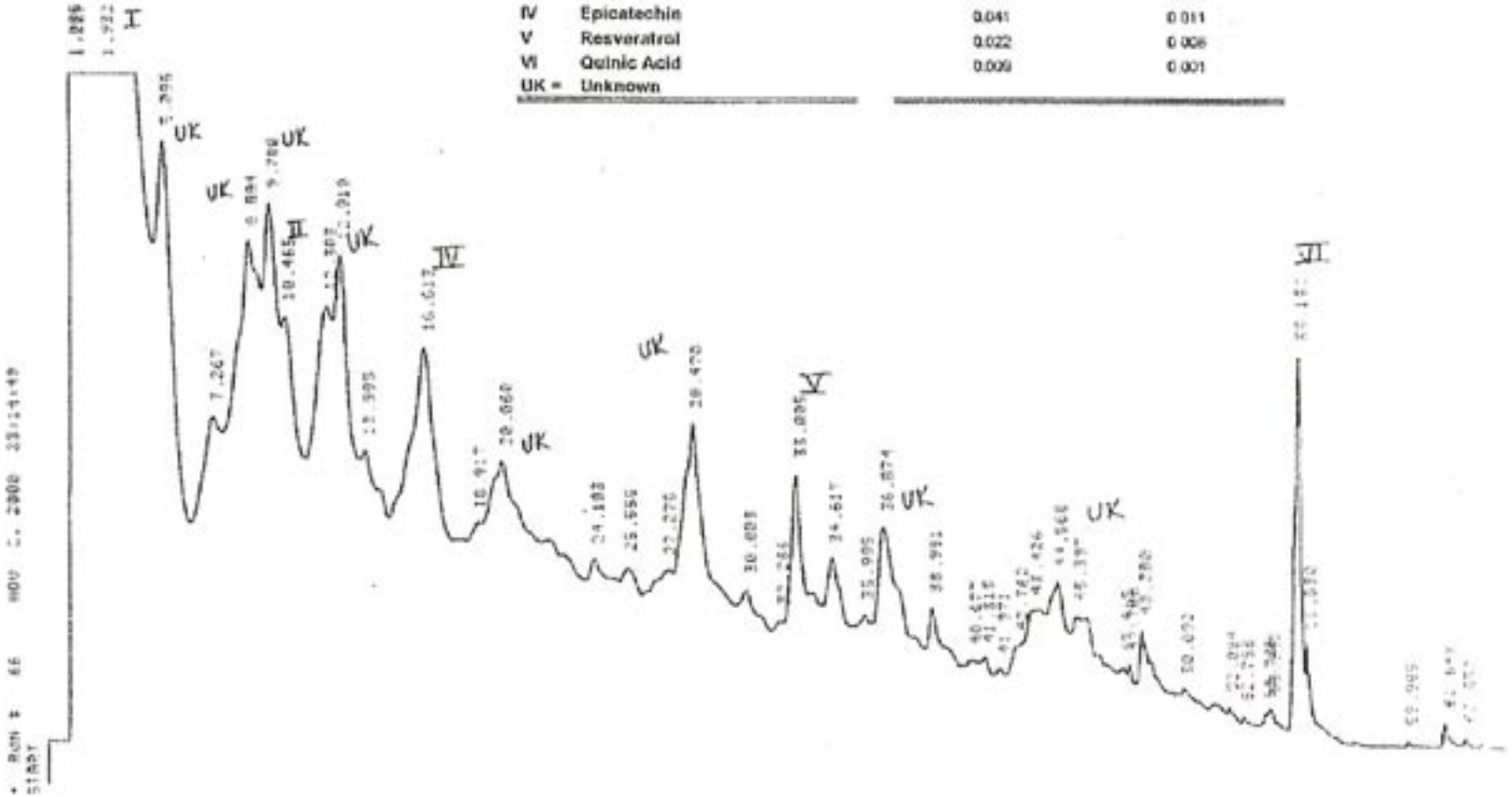


ANTIMICROBIAL ACTIVITY OF THOMPSON, DIPPED, GOLDEN AND ZANTE RAISIN EXTRACTS

We conducted experiments to determine the amount of total phenolics present in different types of raisins and their antimicrobial activity effects in three pathogenic and one non-pathogenic microorganism..

Total phenolic compounds

Four different types of raisins were analyzed. Regular raisins from Thompson seedless grapes (Thompson), raisins exposed to SO_2 while drying (Golden), raisins dipped in sugar solution previous to drying process (Dipped), and raisins from Zante grapes (Zante). Total phenolic content was evaluated by the Folin-Ciocalteau phenol reagent assay (Hyodo et al., 1978; Talcott and Howard, 1999), performing the proper adjustments to apply it to Raisins.

Preliminary test to obtain raisin extracts

Extracts for evaluating the effect on microbial growth were performed in three ways. The phenolic content in raisins was extracted with nanopure water, methanol and also with ethanol, to determine the extraction power of each solvent. Ethanol extracts were not possible to obtain due to a high viscous paste formation. However, using water and methanol gave extracts which were very similar. Both extracts were filtered through a 0.45 μ (methanol) and 5 μ (water) membrane filter.

Antimicrobial properties of raisin extracts (Assay 1)

Raisin extracts were used to test their effect on microbial activity. The microorganisms (MO) used were *Listeria monocytogenes*, *Escherichia coli 0157:H7*, and *Lactobacillus plantarum*. One of the extracts was obtained by homogenizing 5 g of raisins with 10 ml of nanopure water, centrifuged and filtered. The total soluble solid content for the extract was ~ 29.2%. Another extract was prepared homogenizing the tissue with methanol, with the objective of extracting the phenolic content. A total of 20 g of raisins was extracted with methanol, then the solvent was evaporated and the concentrate re-dissolved in water. The solution was passed through Oasis HLB extraction cartridges to eliminate sugars and other water-soluble compounds. The phenolic content was washed out of the cartridge with methanol, methanol was evaporated again and the remaining extract was dissolved in 2 ml of water. The final extract was passed through a nylon 0.45 μm syringe filter and stored in a sterile vial ready for use. A 2% Chlorogenic acid solution was also prepared to compare its effect against the two extracts.

Two microbiology tests were performed to check the activity of the extracts. The Kirby-Bauer Method is usually very good for testing the effects of chemical agents on bacteria. However, it seems to lack sensitivity at the concentrations obtained for the extracts. The test was performed at full strength for three different extract concentrations without showing any significant difference. A more sensitive spectrophotometric test was performed using an automatic densitometer (Bio-Tek Instruments). Full strength was evaluated again with two different media. The test was done in sterile 96-well assay

plates. A 2% peptone water media was not suitable enough for the growth of the microorganisms tested. Mueller-Hinton on the other hand worked fine for all of them. Two concentrations of each microorganism were evaluated in triplicates for each extract, with a control for comparison.

Antimicrobial properties of raisin extracts (Assay 2)

Extracts from 4 different types of raisins were used to test their effects on microbial activity. The microorganisms (MO) used were *Listeria monocytogenes*, *Escherichia coli* 0157:H7, *Salmonella enteritidis* and *Lactobacillus plantarum*. All microorganinsm were tested at two initial microbial concentrations, $10^{\wedge 3}$ and $10^{\wedge 5}$. The extracts were obtained by homogenizing 5 g of raisins with 10 ml of sterilized water, centrifuged and filtered. Since the total soluble solid content for the extracts is ~ 30%, a sugar solution was prepared to test its effect on microbial growth. A sensitive spectrophotometric test was performed using an automatic densitometer (Bio-Tek Instruments). Full strength (1/2) and several dilutions (1/12, 1/120, 1/240) of the extracts were evaluated by following the growth of microorganisms in the presence or absence of extract. Microbial growth was determined based on the appearance and increase of turbidity in the media. The test was done in sterile 96-well assay plates using Mueller-Hinton broth. Two concentrations of each microorganism were evaluated in triplicates for each extract, with a positive and a negative control for comparison.

Results

Total phenolic compounds

The total amount of phenolics was higher for Golden raisins, followed by Zante and Thompson raisins (Table 1). These results confirm that depending on how Thompson seedless grapes are processed, the amount of phenolics will differ and most likely also their functional properties such as antimicrobial activity. Golden raisins had in average 67.4% more phenolic compounds compared to Thompson raisins and 83.5% more compared to Dipped raisins. The difference observed is likely due to browning reactions occurring during the drying process mediated by Polyphenoloxidase (PPO), causing polymerized brown pigments that give raisins their color. Zante raisins, which come from red grapes, have higher levels of phenolics compared to Thompson raisins and Dipped raisins. However, results indicate that Golden raisins have 31.1% more phenolic compounds than Zante raisins. Again, we observe that the processing method to obtain raisins would be determinant in the final content of total phenolics.

Table 1. Total phenolic content of raisins

PRODUCT	Phenolic Content (mg/100g)			Avg Phen. Content	Stadev
	R1	R2	R3	mg/100g	
Thompson	435.3	401.7	594.9	**477.3**	**103.2**
Golden	777.1	770.9	850.2	**799.4**	**44.1**
Dipped	433.8	465.3	407.6	**435.5**	**28.8**
Zante	576.4	687.7	564.3	**609.5**	**68.0**

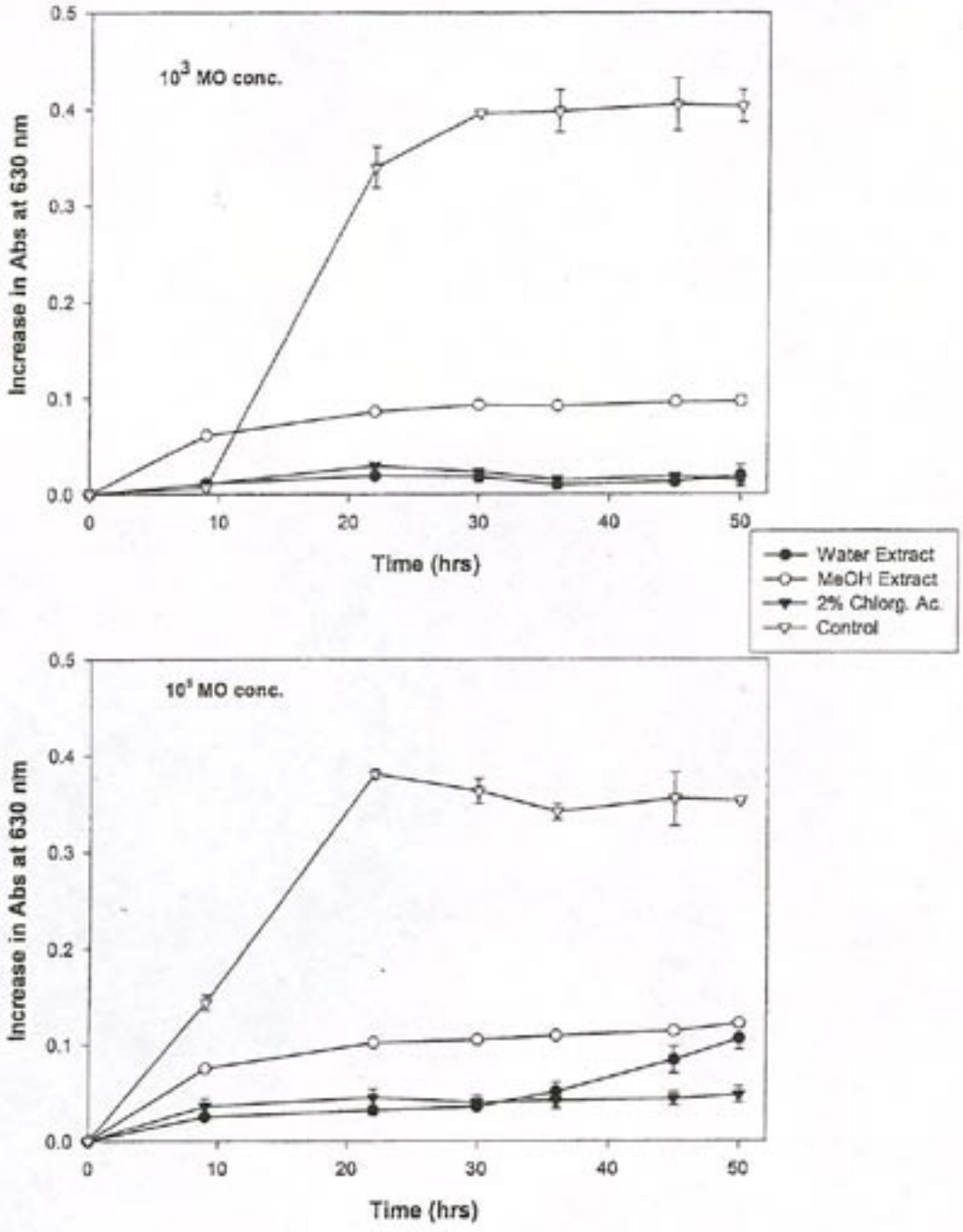
Antimicrobial properties of raisin extracts (Assay 1)

All extracts were very good at inhibiting the growth of *L. monocytogenes* compared to the control for both MO concentrations used. The water extract was very similar in activity to the 2% chlorogenic acid solution keeping the microbial growth 80-95% lower than the control. The MeOH extract showed about 70% inhibitory effect on *Listeria*. In the case of *E. coli*, the effect for all extracts was similar for an initial 10^5 MO concentration, giving ~ 60-70% inhibitory effect. At the lower initial 10^3 MO concentration, the water and the 2% chlorogenic acid extract had an approximate 80% inhibitory effect, while the MeOH extract showed ~50%.

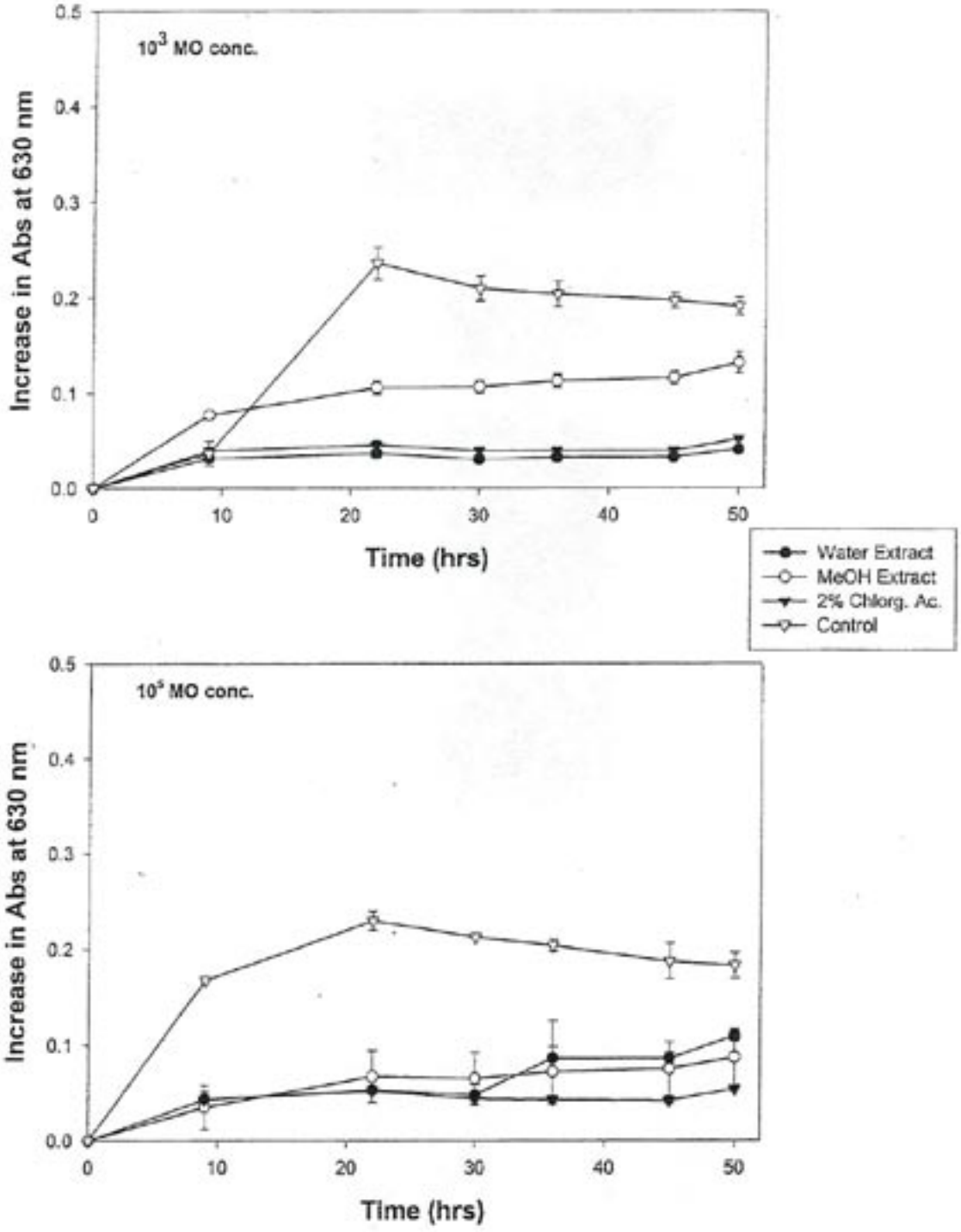
Extracts acted different upon *L. plantarum* compared to *E. coli* and *L. monocytogenes*, even the control showed lower microbial growth for the length of the experiment. MO's growing in the water extract had growth rates similar to the other extracts for the first 20 hrs. After that lag phase MO's started growing faster under the water extract and increased by 70-75% compared to the other treatments. The 2% chlorogenic acid solution was the only treatment that worked better on inhibiting *L. plantarum*. The MeOH extract activity was very similar to the control at the end of the experiment..

All extracts showed very good inhibition on L. monocytogenes and E. coli, both human pathogens. This could be due to the presence of the phenolic substances or the effect of high sugar concentration in the water extract, which can lower the water activity of the media. However, our measurements of water activity showed values of 0.95 that indicates no water activity inhibition effect. Results with *Lactobacillus plantarum* could be explained from the point of view that these lactic acid bacteria use sugars as a main source of substrate. Therefore, it could be able to grow better in sugar-rich media and the water extract might provide the substrate.

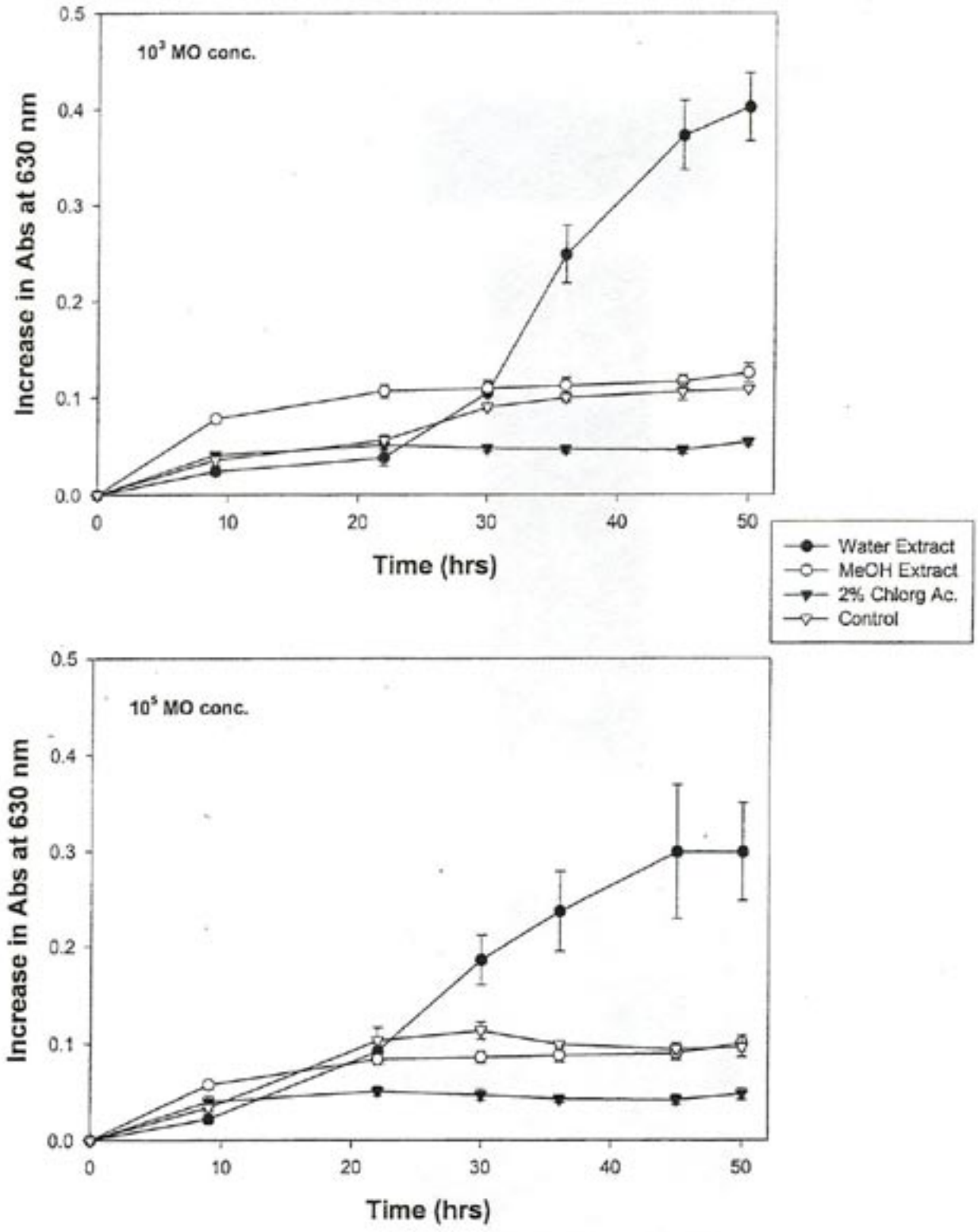
Listeria Monocytogenes



E. coli


10^3 MO conc.
Increase in Abs at 630 nm
Time (hrs)
Water Extract
MeOH Extract
2% Chlorg. Ac.
Control
10^5 MO conc.
Increase in Abs at 630 nm
Time (hrs)

Lactobacillus plantarum



Antimicrobial properties of raisin extracts (Assay2)

The amount of phenolic compounds in the raisin extracts was determined from Table 1 and with the dilution factors used in the antimicrobial assay. Thus, for full strength Golden raisins we estimated ~ 399.7mg/100g extract, Thompson, ~ 238.6mg/100g, Dipped raisins, ~ 217.7mg/100g, and Zante, ~ 304.7mg/100g.

The first experiment was done to determine if the amount sugar present in the extracts (~30% in full strength) and its dilutions could inhibit the growth of the microorganism used in this study. We observed no inhibition for any microorganism tested at both initial microbial concentrations. For example, we observed no inhibition in *L. monocytogenes* growth rate.

In a second experiment, we tested different raisin extracts and observed very good growth inhibition for most microorganisms tested and at both MO concentrations used. The inhibition effect was mainly at the full strength dilution (1/2) extract. At higher extract dilutions (1/12. 1/120, 1/240) slight or non-inhibitions were observed. We only report results for MO concentration of $10^{\wedge 5}$ to show these effects. At $10^{\wedge 3}$ we obtained similar results.

Table 2. *Microorganism growth inhibition or growth enhancing effects of full strength raisin extracts after 48 hrs at 30 C compared to a control.

Raisin extract	*E. coli*	*S. enteriditis*	*L. monocytogenes*	***L. plantarum***
Thompson	- 65%	- 64%	- 63%	+ 130%
Dipped	- 82%	- 75%	- 87%	+ 86%
Golden	- 89%	- 90%	- 84%	- 83%
Zante	- 59%	- 56%	- 56%	+ 60%

***$10^{\wedge 5}$ initial MO concentration**

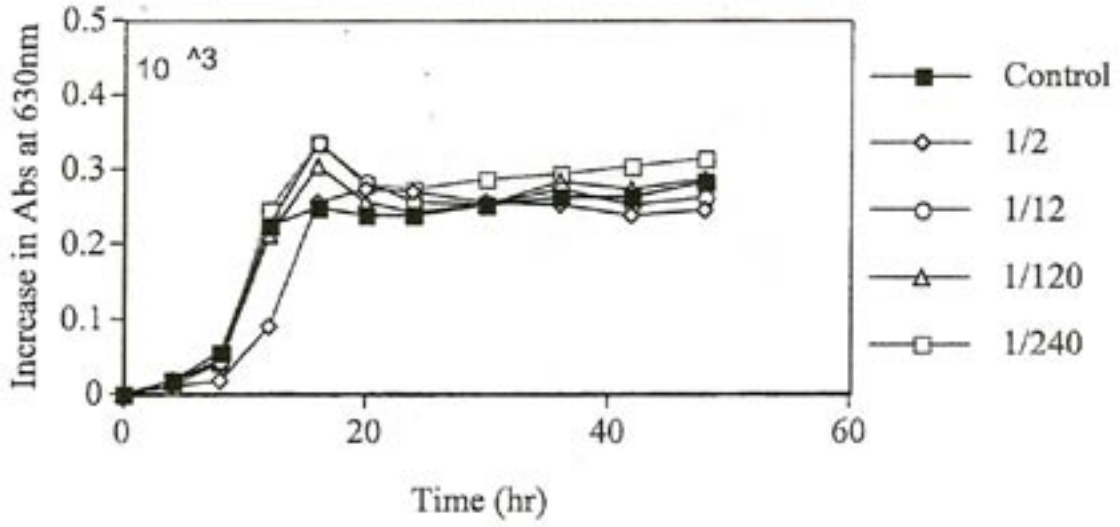
In general the results indicate a consistent overall inhibition effect over pathogenic gram-negative microorganisms (See Figures and Table 2). For each type of raisin extract the inhibition effect is similar across the different MO tested. However, we observe that among raisin extracts there are large differences in inhibition effects. For example, Thompson, Dipped, Golden and Zante raisins kept *E. coli* microbial growth 65, 82, 89 and 59% lower than the control, respectively (Table 2). Golden raisins have the largest inhibition effect compared to the other raisins. This could be due to the larger amount of phenolic compounds present in the extract (Table 1). However, Dipped raisins also show a large inhibition effect, but the amount of phenolic is much lower. Thus, most likely the differences could be due to the type of phenolic compounds present in each raisin.

For gram-positive non-pathogenic *L. plantarum*, raisin extracts acted differently. Thompson, Dipped and Zante raisin extracts enhanced microbial growth ranging from 60 to 130% growth increase compared to the control. Most likely, the sugars present in the extracts were used as a source of substrate for growth. However, Golden raisins did have a large growth inhibition effect over *L. plantarum*. This unexpected result could be related to the type of phenolic compounds still remaining in Golden raisins. The Golden

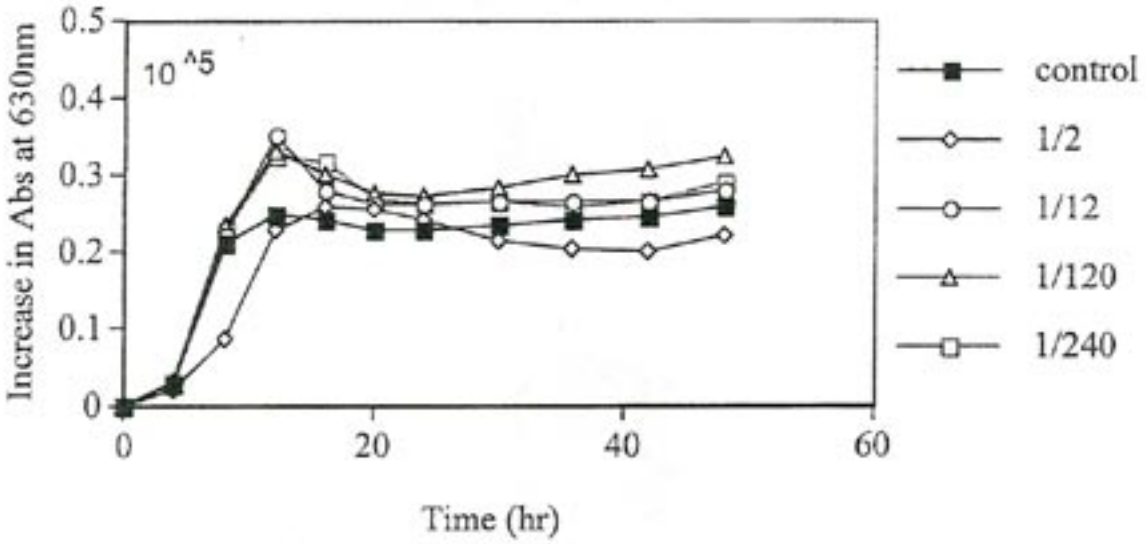
raisin making process uses SO_2 while drying, which could protect some of the phenolic compounds from degradation.

These results confirm that raisins have an inhibitory effect on microbial growth. Raisins, according to our results, can be a potential source of natural microbial growth inhibitors of *E. coli*, *S. enteriditis* and *L. monocytogenes*. Golden raisins seem to have the largest antimicrobial effects and could be considered a good candidate for its use in the food industry as natural antimicrobials. Additionally, these results would indicate the need in having appropriate raising making processes to avoid degradation of important bio-active ingredients, such as phenolic compounds.

L. monocytogenes and sugar solution

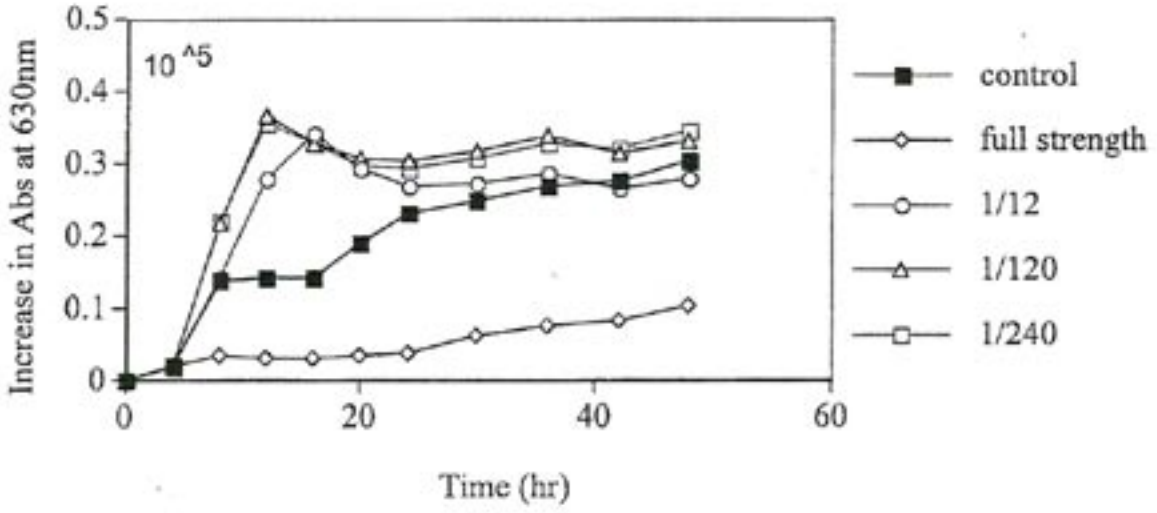


L. monocytogenes and sugar solution

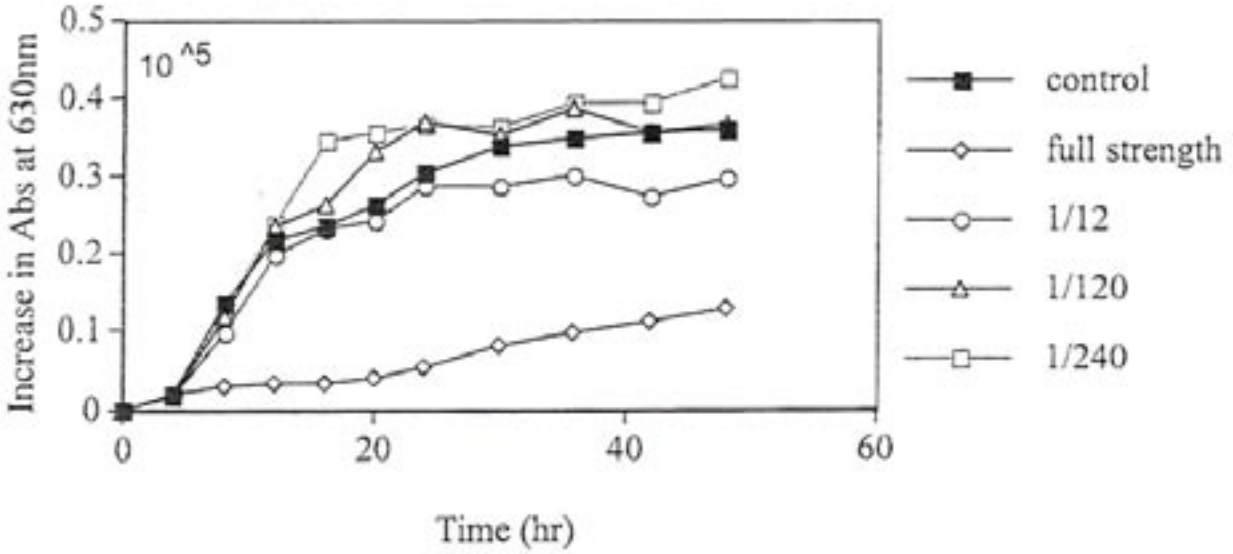


Antimicrobial effects of raisin extracts

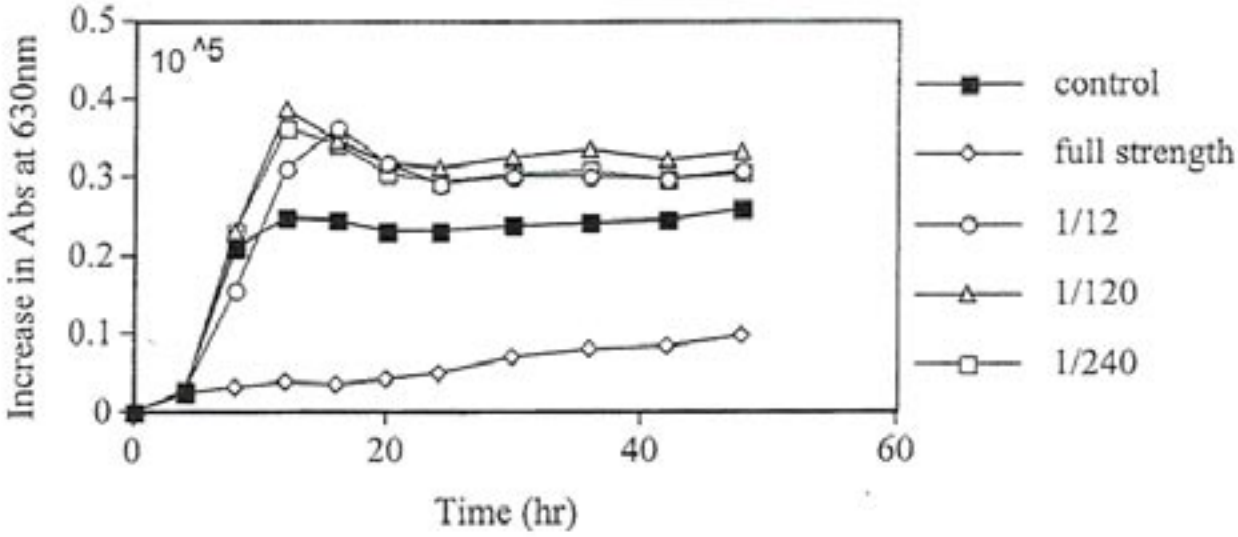
E. coli and Thompson raisin extracts



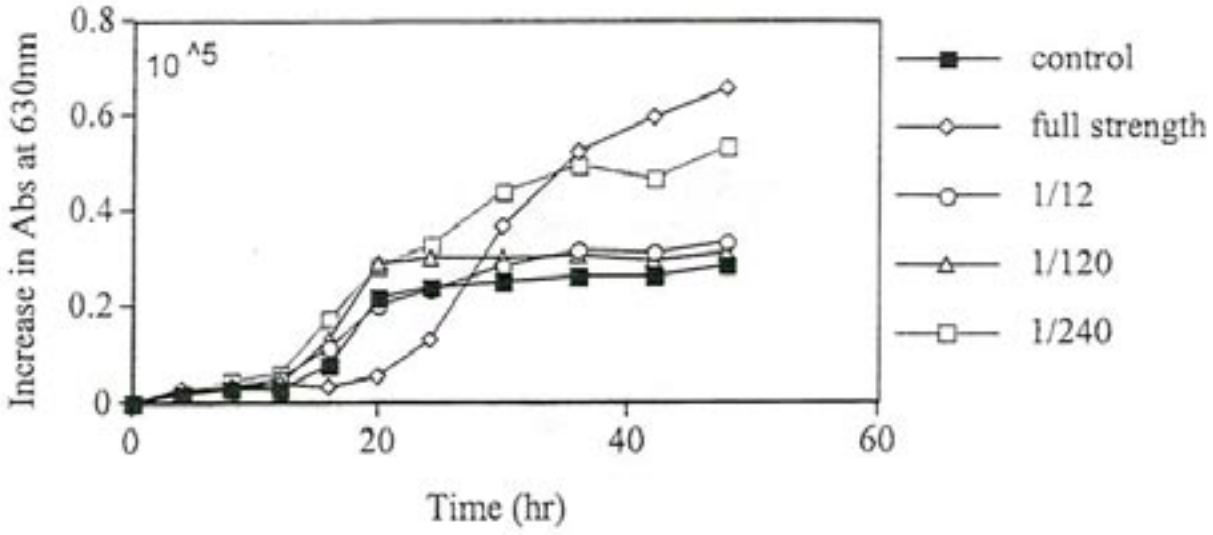
S. enteriditis and Thompson raisin extracts



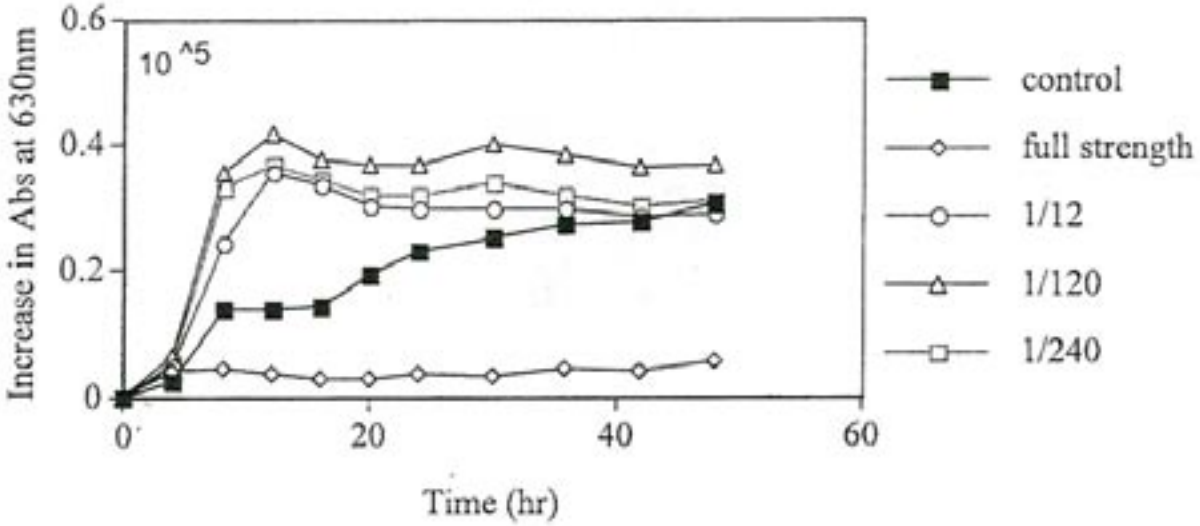
L. monocytogenes and Thompson raisin extracts



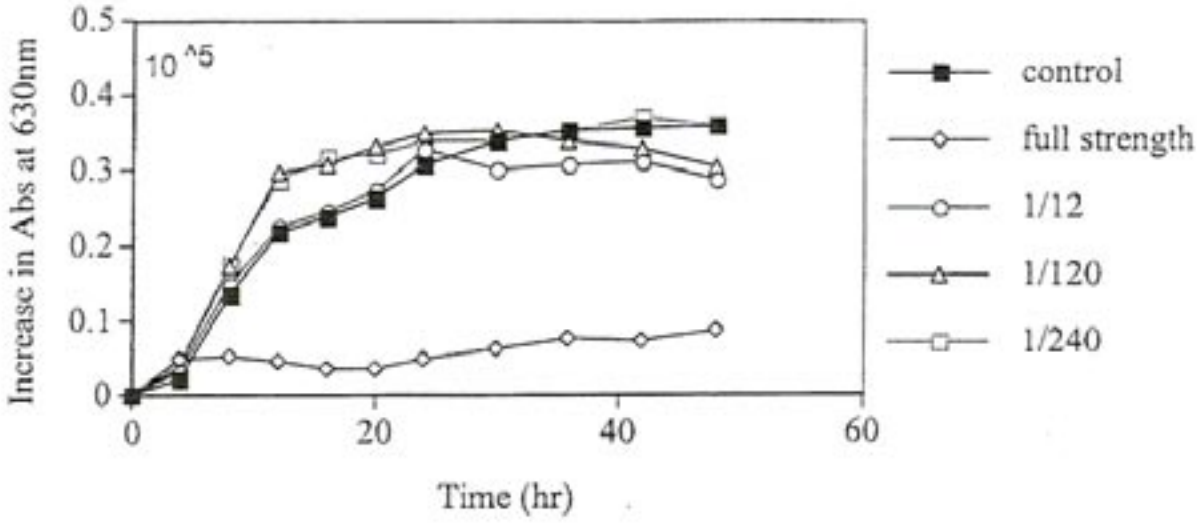
L. plantarum and Thompson raisin extracts



E. coli and Dipped raisin extracts



S. enteriditis and Dipped raisin extracts



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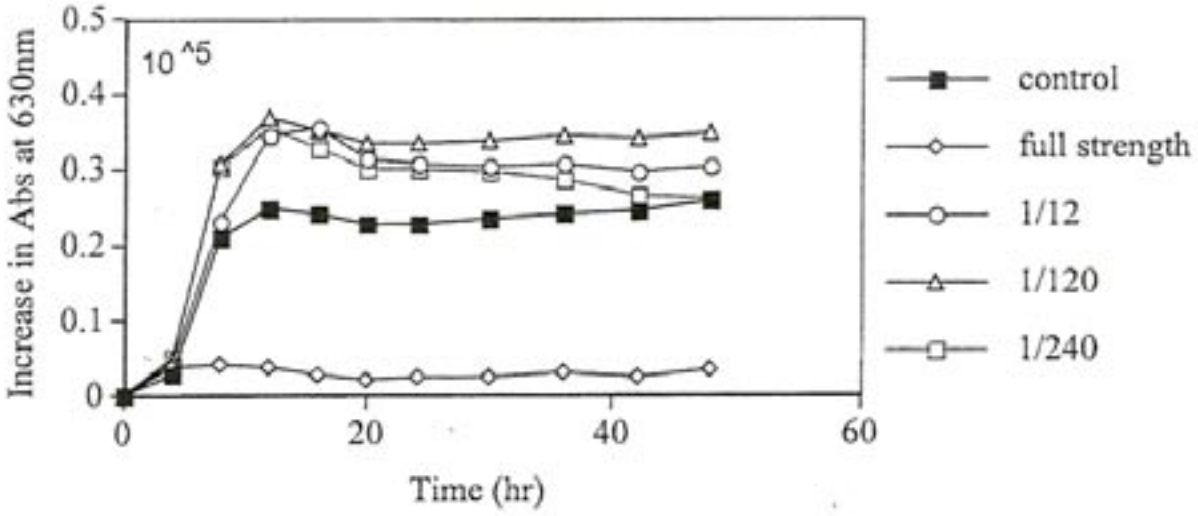

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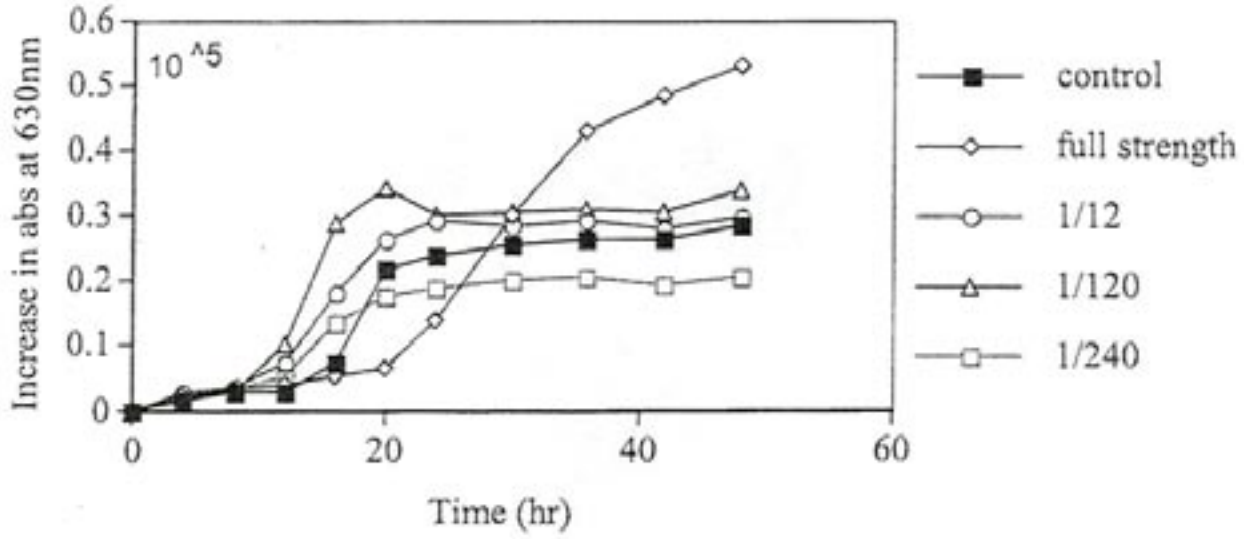

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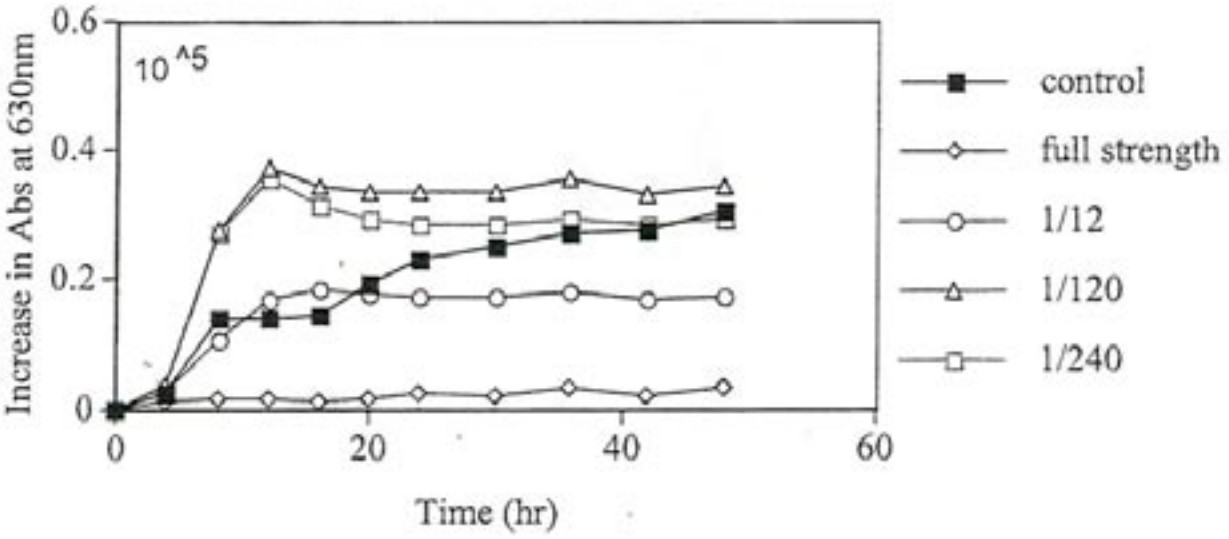

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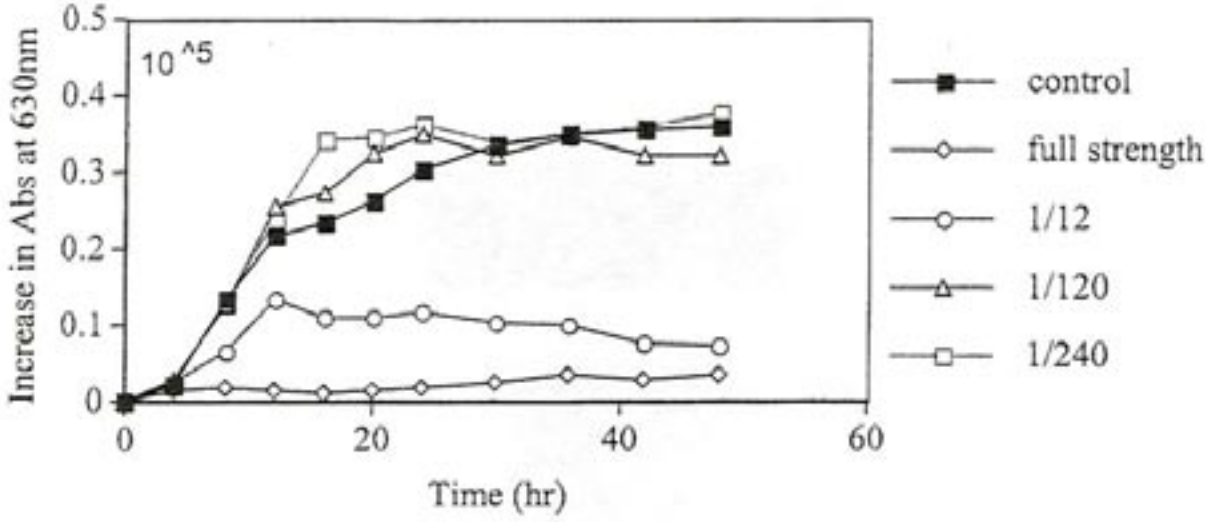

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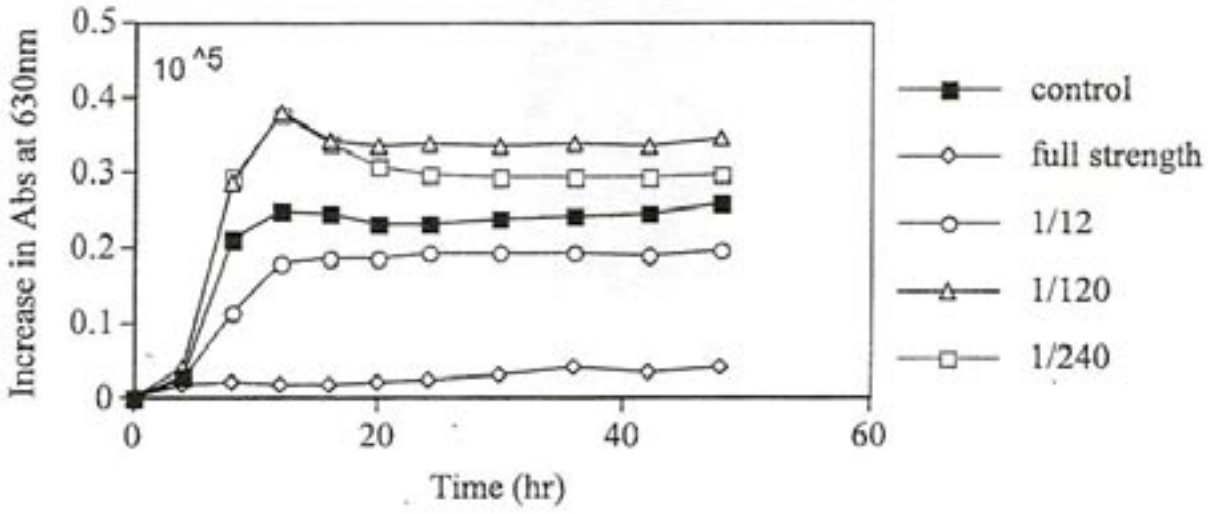

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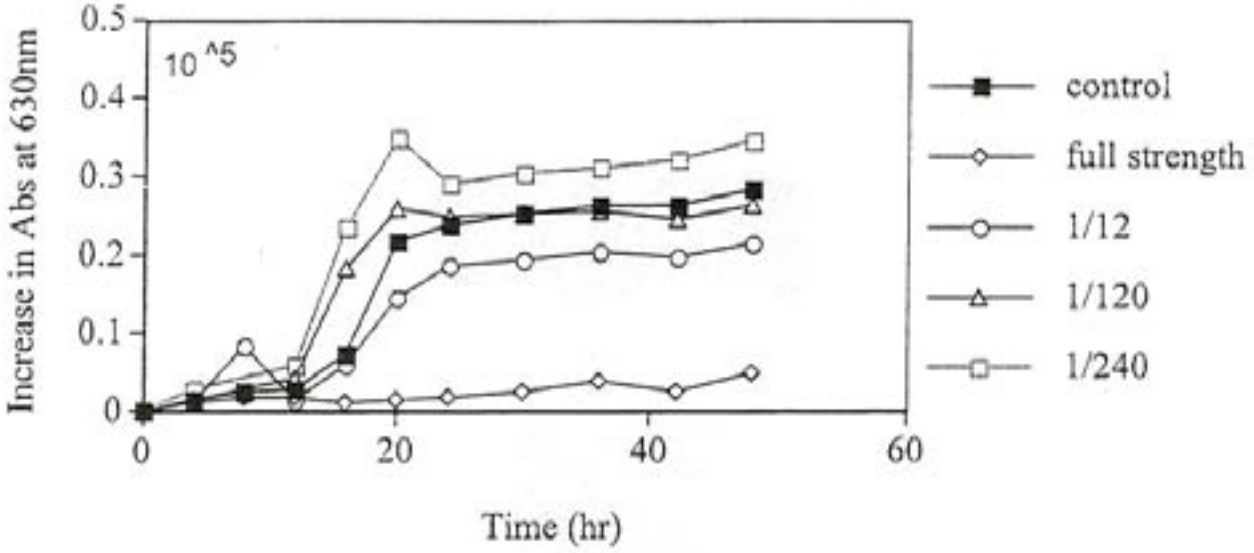

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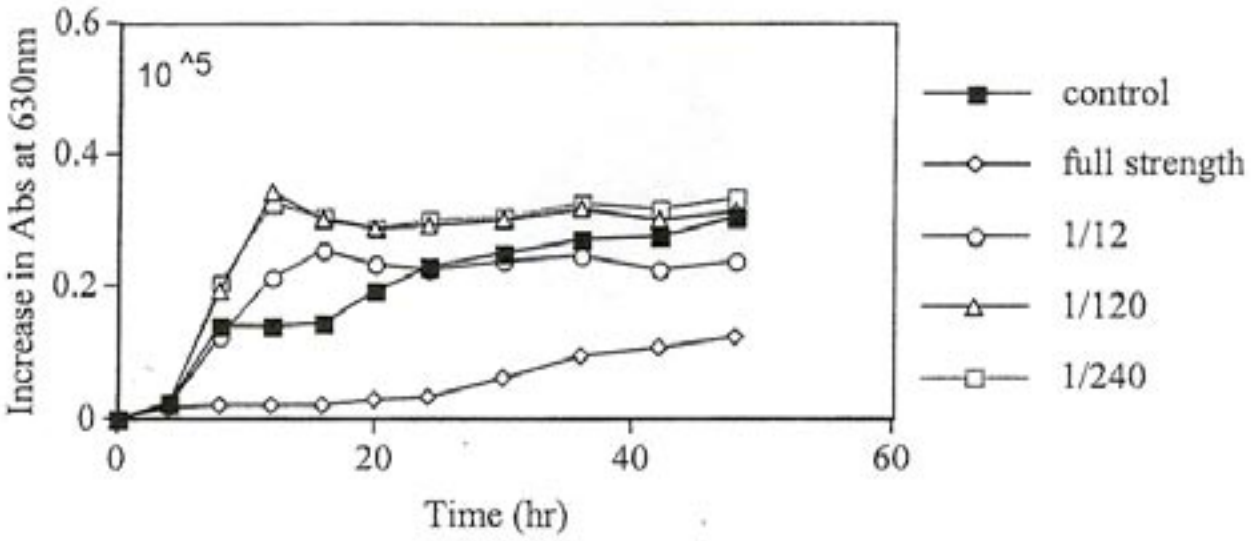

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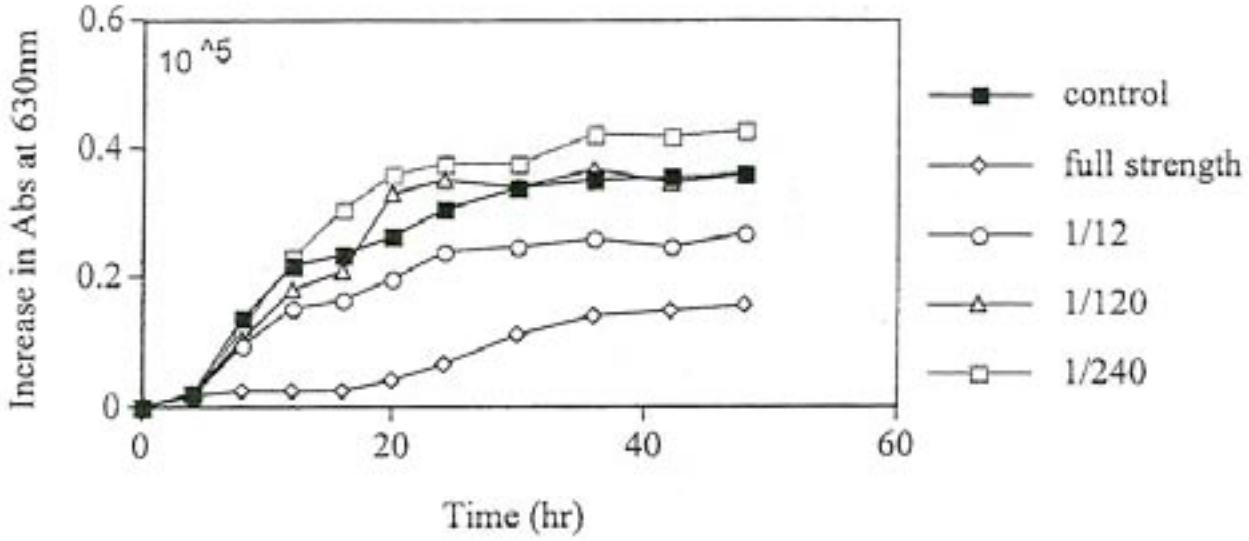
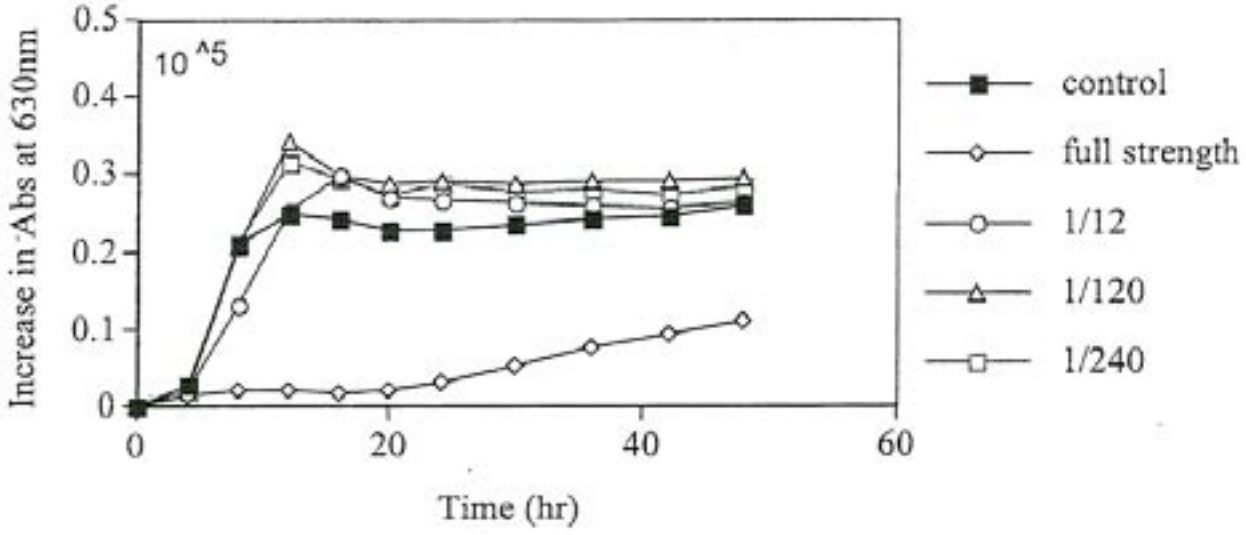
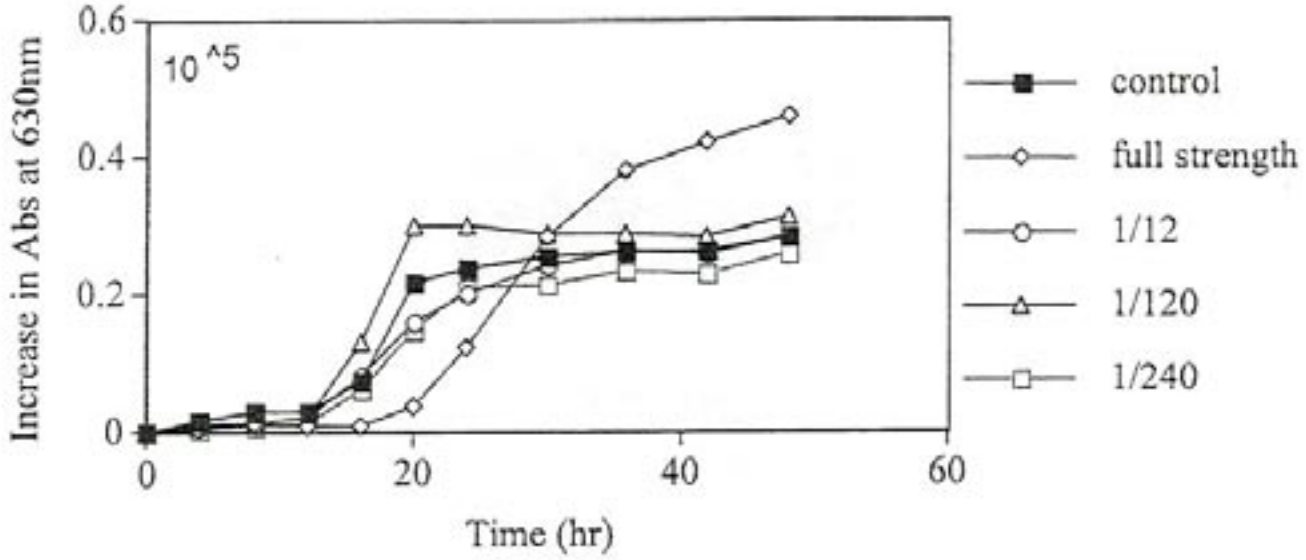


L. monocytogenes and Zante raisin extracts



L. plantarum and Zante raisin extracts



USE OF RAISIN EXTRACTS TO PREVENT BROWNING OF FRESH-CUT APPLES

We conducted experiments to determine the effect of raisin extracts in reducing browning discoloration in fresh cut apples, and the antioxidant activity of different types of raisins.

Material and methods

Apples were obtained from a local supermarket and used immediately for all experiments. Three different types of apples were tested, Red delicious, Golden delicious, and Granny smith. Red delicious seemed to undergo browning faster compared to the other two types of apples, therefore Red delicious were used to further study the effect of raisin extracts. Full strength (100%) raisin extracts were prepared buy homogenizing 10 g of raisins with 20 ml of nanopure water, filtered through cheese cloth and then centrifuged at 14 500 rpm for 15 min. Proper dilutions were made out of the full strength solution to get the required strengths of 25% and 50%. Pieces (disks 11 mm diameter x 5 mm thickness) of apples were cut out of whole apples and immediately placed into 4 different treatments, control (0%), 25%, 50%, and 100% strength of raisin extracts for both Thompson (sun-dried) and Golden raisins. Nanopure water dips were used as control. The extracts were applied by dipping the apple disks in the different treatments for 3 min, blotted carefully to eliminate excess water, and immediately read in a HunterLab color meter (Labscan SN-12384). Readings were taken at different time intervals, along with pictures. Due to water loss, the experiment was extended for only 4 hours at 20 °C. At this time the water loss was being very evident at the storing temperature.

Color parameters used were L*, a*, b*, Chroma and Hue values. The L* indicates color lightness, a* is the scale for color from green (-) to red (+), b* is the scale for color from blue (-) to yellow (+) and chroma values are the actual color of the subject given be the relationship Chroma = $(a^{*2} + b^{*2})^{0.5}$. The Hue value is the angle formed between a* and b* components of color and is given by the following relationship, Hue = Arctangent b*/a*

Antioxidant assay

Extracts from Golden, Dipped, Thompson seedless and Zante raisins were allowed to react with a stable radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) in a methanol solution (Brand-Williams et al., 1995). In it's radical form, DPPH• absorbs at 515 ηm, but upon reduction by an antioxidant (AH) or a radical species (R•), the absorption disappears

$$DPPH\bullet + AH \text{ ---------- } DPPH\text{-}H + A\bullet$$
$$DPPH\bullet + R\bullet \text{ ---------- } DPPH\text{-}R$$

The use of DPPH• provides an easy and rapid way to evaluate the antiradical activities of antioxidants. We prepared two standard curves, one with Ascorbic Acid (vitamin C) and one with Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a very

powerful antioxidant), to use as a reference with our extracts. We also evaluated the antioxidant activity of blueberries to establish a comparison with our raisin's extracts.

Results

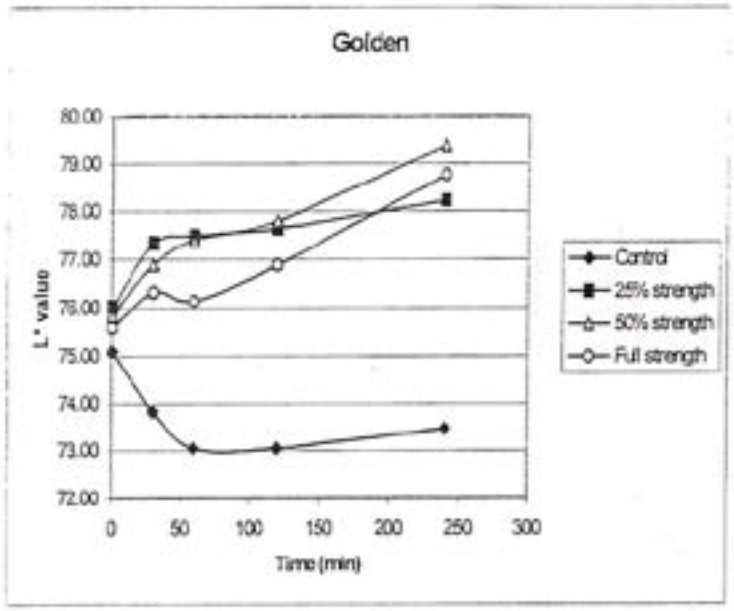
The experiment was performed to see the effect of Raisin extracts on the browning reactions of Apples. Figures from apple experiments show that both Thompson (sun-dried) and golden raisins extracts helped to prevent browning, but at different level. Golden raisins extracts at all concentrations were better at preventing the browning of fresh cut apples than Thompson raisin extracts as it can be seen in the figures. In the case of Thompson raisin extracts, the effect on preventing browning was less noticed due to the brown color already present in the extract. The extract actually conferred some slight brown pigmentation to the apple disks and the intensity was in accord with its strength. For that reason, it seems that the less colored extract (25% strength) showed better results on preventing browning. This effect can also be seen in the pictures. Pictures for Golden raisin extracts were taken at the start of the experiment and 4 hours after, and for Thompson (sun-dried) raisin extracts pictures show effects after 1 and 4 hours, respectively.

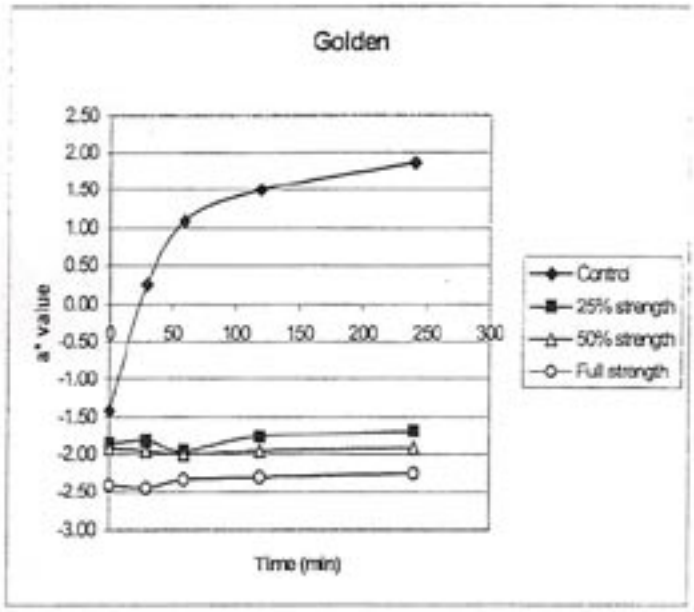
For the Golden raisin extracts, all color parameters such as L*, a*, b*, chroma, and hue values were retained or improved when compared to the control. The efficiency of the different concentrations of raisin extract (strengths) on preventing browning was very similar among them, observing only slightly differences. It can be seen in the pictures too. Something that could be needed to elucidate is if the anti-browning effect is due to some antioxidant or reducing agent in the extract, or any residual concentration of SO_2 from the SO_2 treatment when making the Golden raisins. However, the fact that Thompson raisin extracts also helped to delay browning suggests that there might be a natural anti-browning agent.

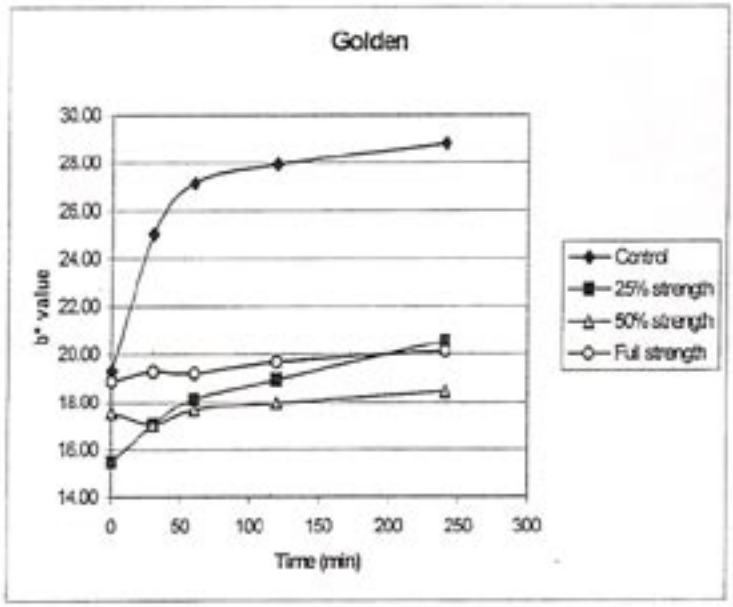
The antioxidant assay showed that Golden raisins had higher antioxidant activity than Thompson, Dipped and Zante raisins. These last three raisins had similar activity. The difference in activity observed may explain the actual anti-browning effects these two raisins have in apple tissue. When compared to Vitamin C, Golden raisins show a 99.5% of the activity of this antioxidant in blueberries. And when compared as Trolox, Golden raisins again show 99.5% of that in blueberries.

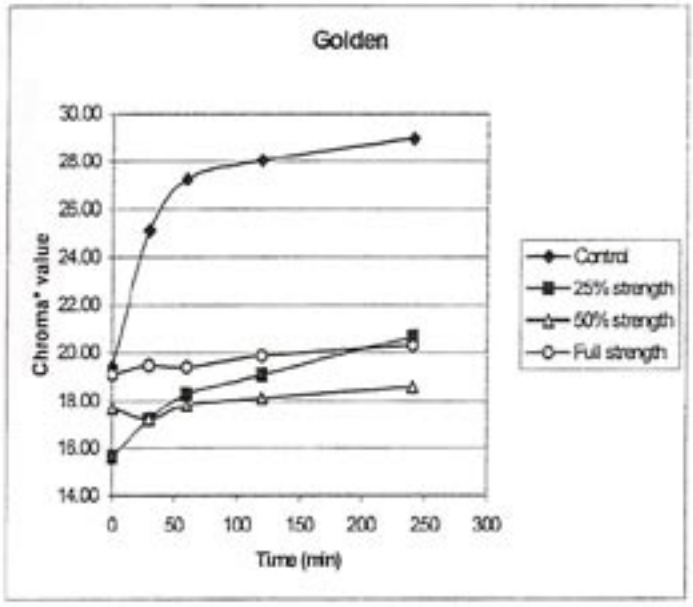
Table 1. Antioxidant activity of raisins

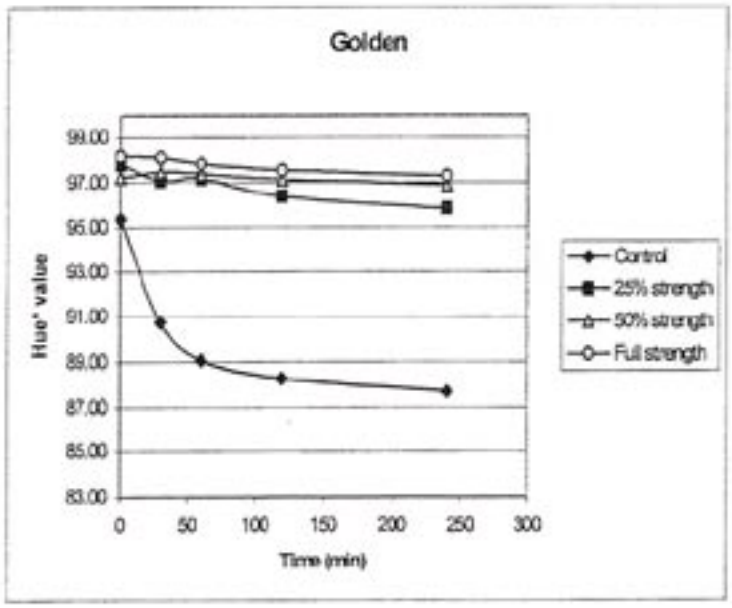
PRODUCT	Ascorbic Ac Equivalent (µg/g of raisins)	Stadev	Trolox Equivalent (µg/g of raisins)	Stadev
Thompson	446.66	25.14	723.31	39.13
Golden	1509.28	222.17	2377.19	345.80
Dipped	425.64	119.04	690.60	185.28
Zante	485.29	25.05	783.45	38.99
Blueberries, TX	1516.58	294.38	2390.51	457.94

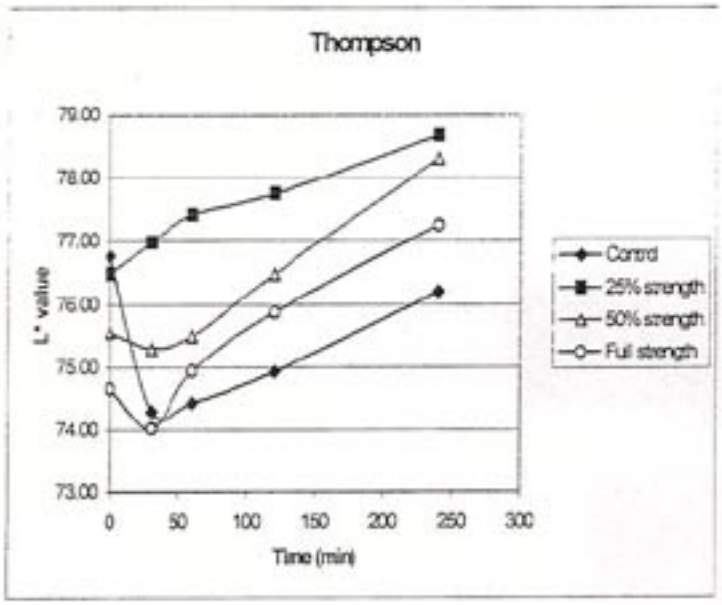

Golden
L* value
Time (min)
Control
25% strength
50% strength
Full strength

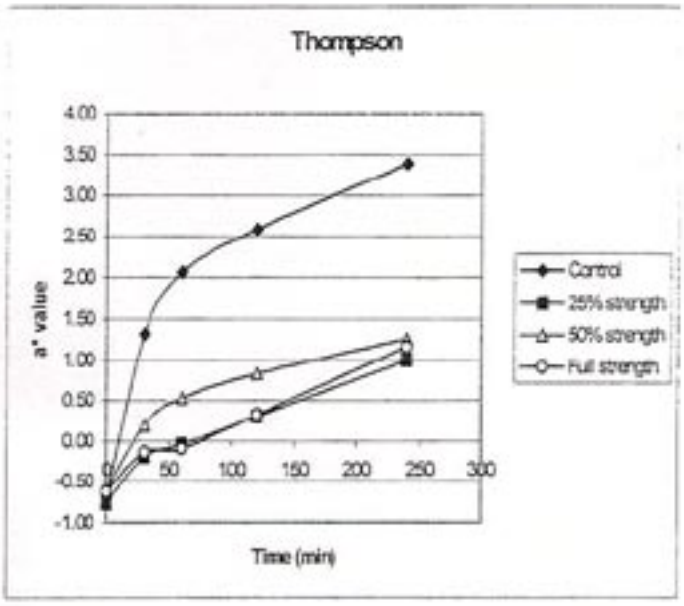

Golden
a* value
Time (min)
Control
25% strength
50% strength
Full strength

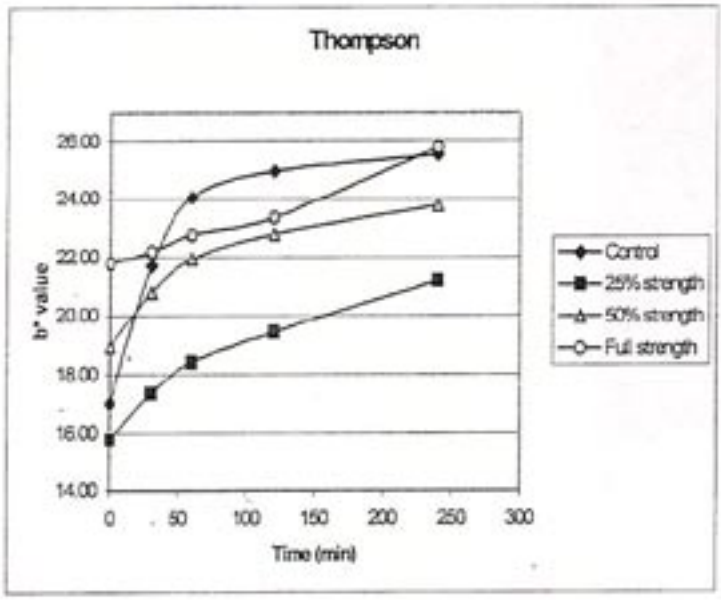

Golden
b* value
Time (min)
Control
25% strength
50% strength
Full strength

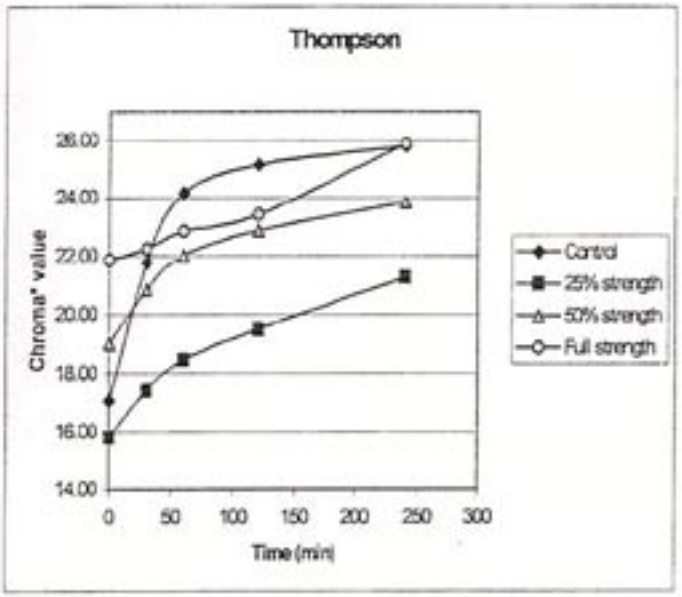

Golden
Chroma* value
Time (min)
Control
25% strength
50% strength
Full strength

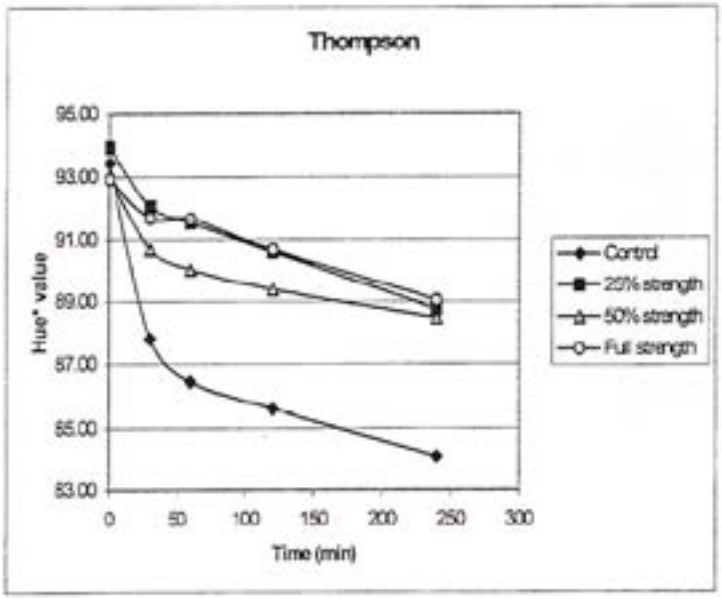

Golden
Hue* value
Time (min)
Control
25% strength
50% strength
Full strength

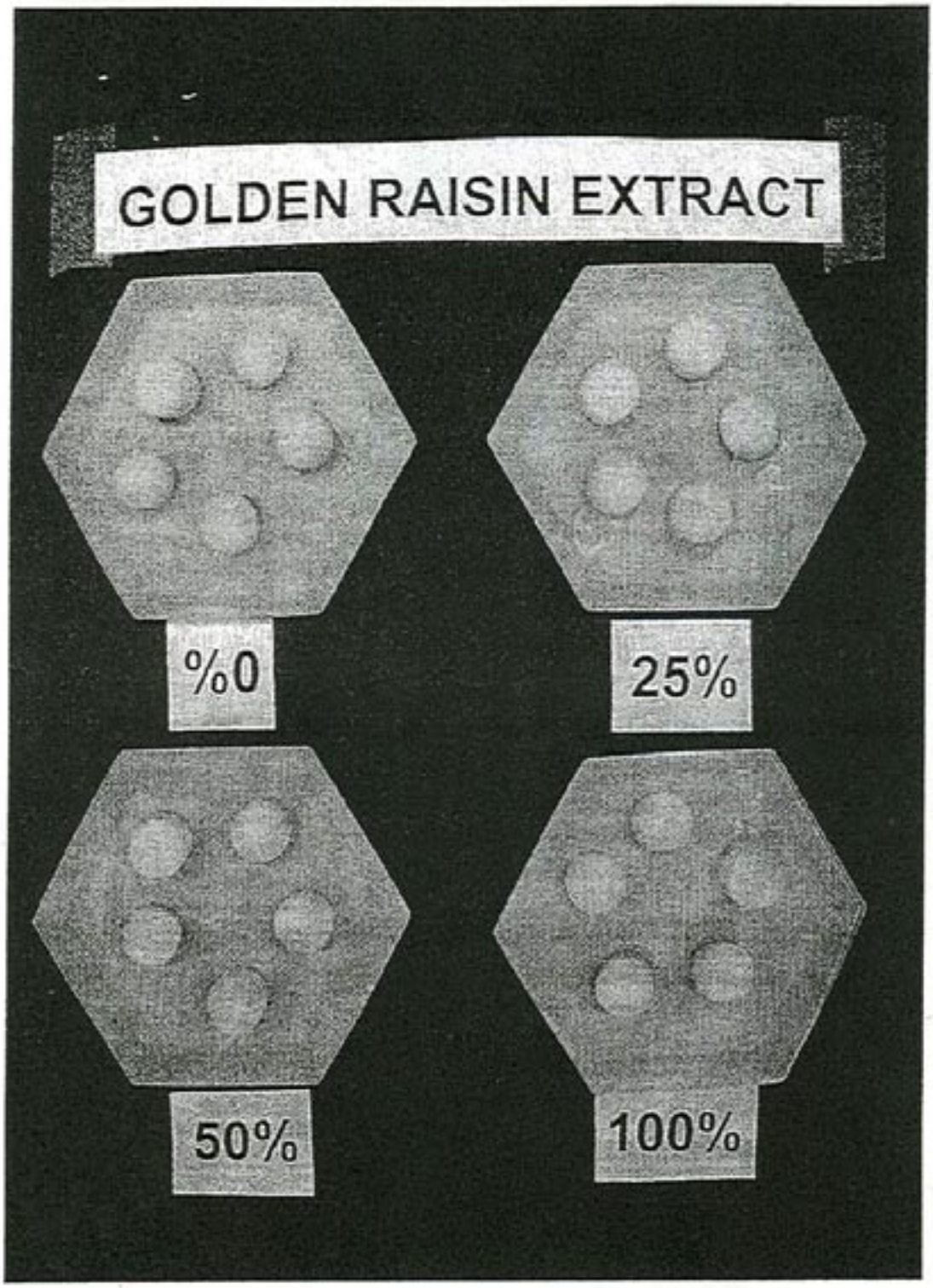

Thompson
L* value
Time (min)
Control
25% strength
50% strength
Full strength


Thompson
a* value
Time (min)
Control
25% strength
50% strength
Full strength

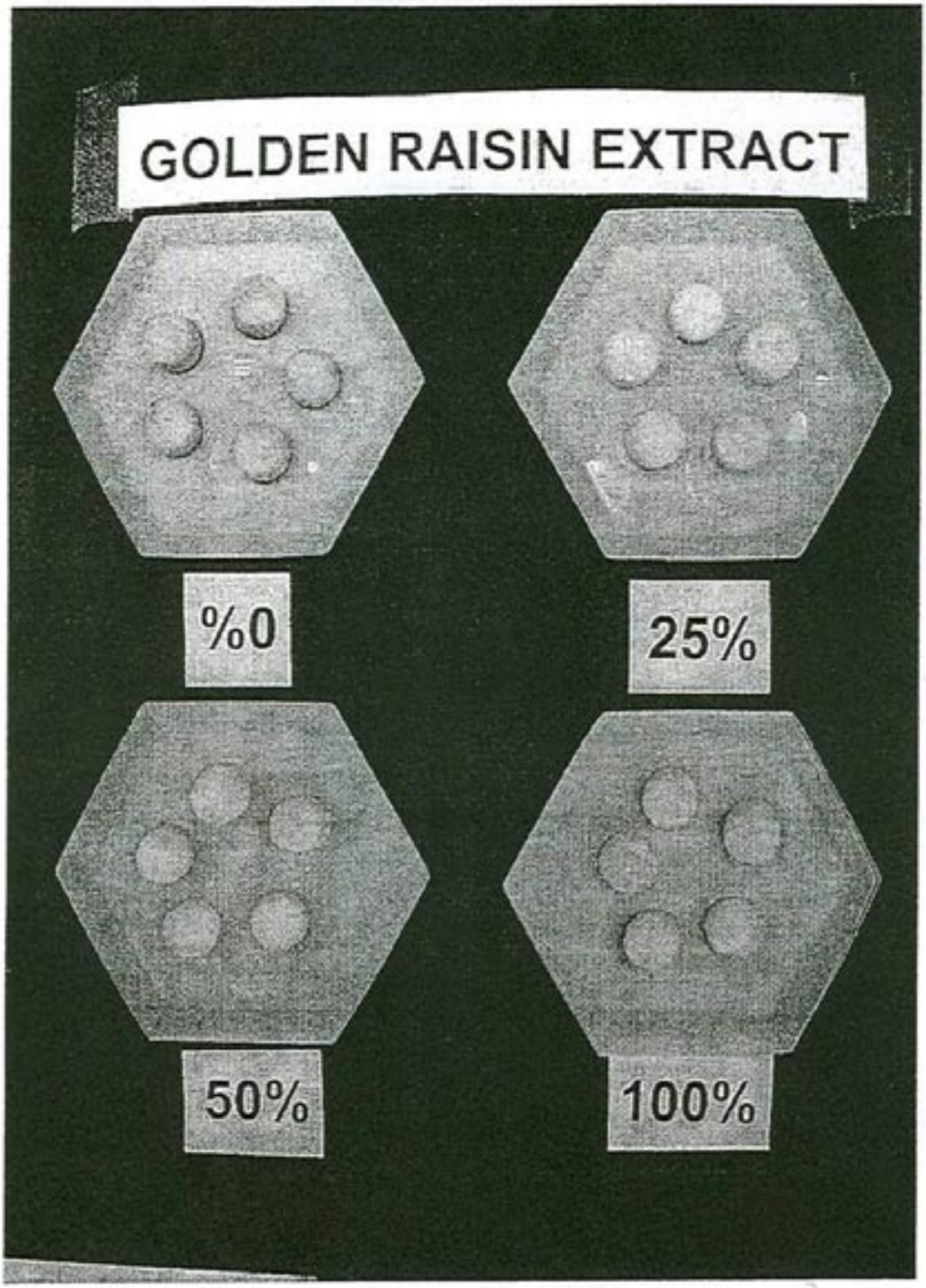

Thompson
b* value
Time (min)
Control
25% strength
50% strength
Full strength


Thompson
Chroma* value
Time (min)
Control
25% strength
50% strength
Full strength

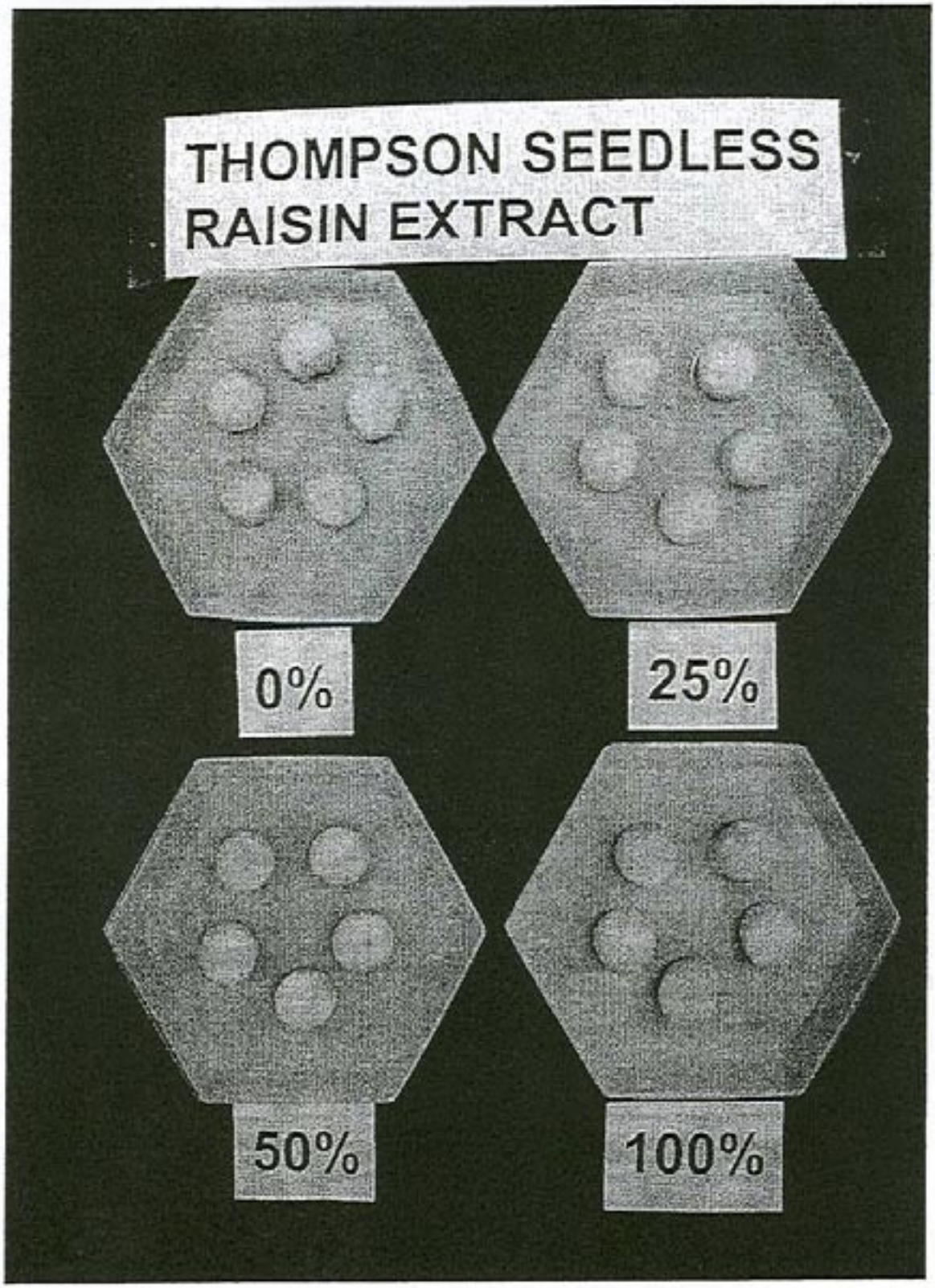

Thompson
Hue* value
Time (min)
Control
25% strength
50% strength
Full strength



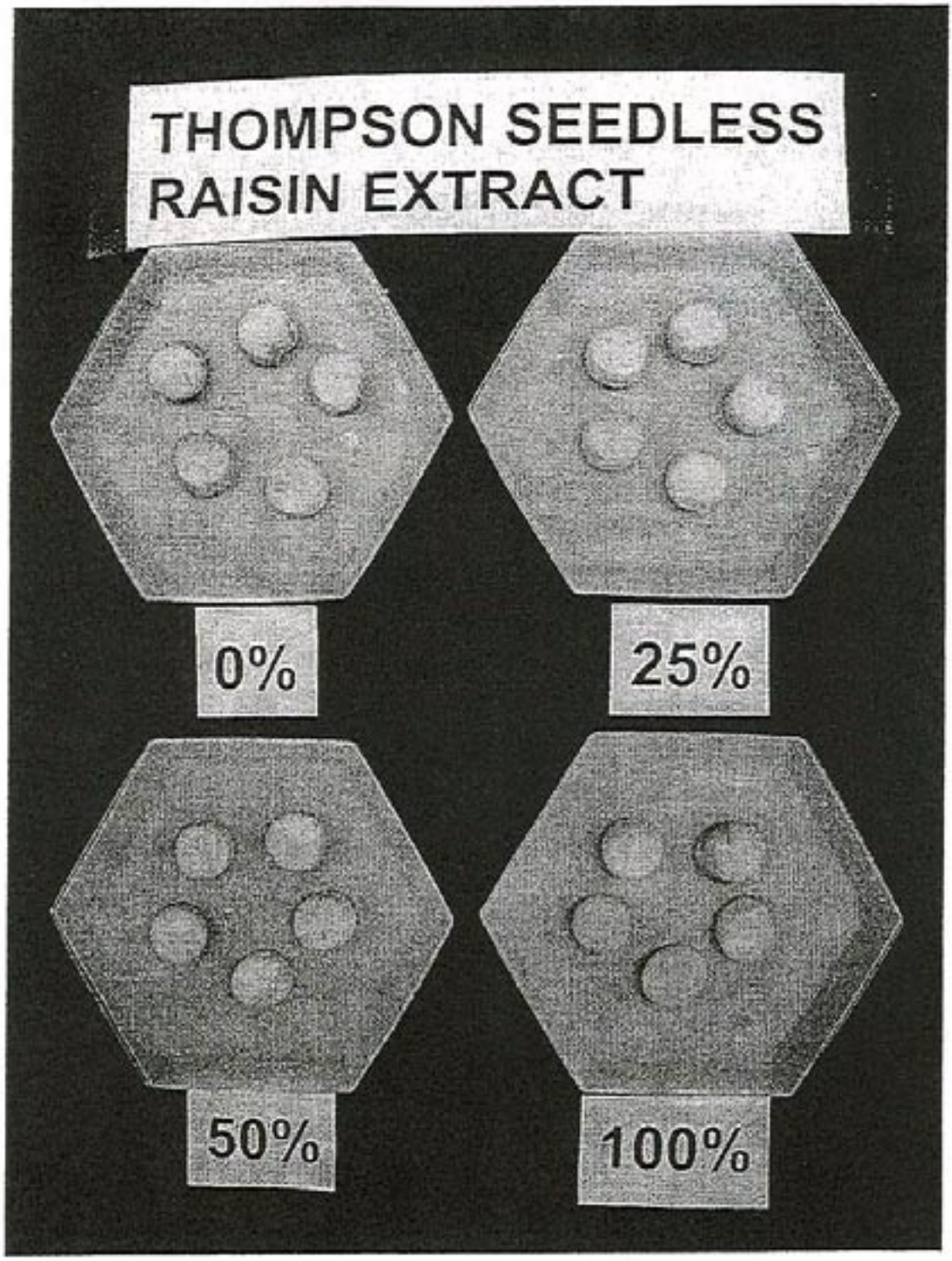
Apple pieces dipped in Golden raisin extracts: Initial Time



Apple pieces dipped in Golden raisin extracts: After 4 hrs



Apple pieces dipped in Thompson seedless raisin extracts: After 1 hrs



Apple pieces dipped in Thompson seedless raisin extracts: After 4 hrs

USE OF RAISIN EXTRACTS TO PREVENT MICROBIAL GROWTH ON FRESH-CUT PRODUCE.

Material and methods

Apples, lettuce, cantaloupe melons, carrots and celery were obtained from a local supermarket and tested to see if they made a good media for *Listeria monocytogenes* to grow. Apple's pH was too low to allow the bacteria growth, lettuce were complicated to set a nice model to work with, and carrots are known to have natural inhibitors that prevent the growth of *Listeria*. Celery and Cantaloupe melons worked better for performing fresh-cut operations and setting up a system to work on. Fruit disks of 11 mm diameter (cantaloupe melons) and sticks (celery) with enough thickness to give pieces of ca 2.5 g were cut and washed in chlorinated water (50 ppm) to eliminate any initial microbial load. Fresh cut melons and celery sticks were let to stand at 2.5 °C for a few hours to allow chlorine to evaporate. Several tests were run to determine the appropriate concentrations of bacteria to inoculate on the fresh cut produce. To make bacteria load homogeneous on the product, melon and celery pieces were inoculated with 10 µL of bacteria solution (105 and 107 cfu/ml concentrations). Bacteria were allowed to acclimate for 24 hours on the product. After acclimation, melon disks and celery sticks were sprayed with full-strength extracts of Thompson and Golden raisins, and water as a control. Fresh cut pieces were stored at 5 °C and samples were collected at initial time and 8 days after the experiment started. Samples (5g) were homogenized with 45 ml of 0.1% peptone water and 100 µL aliquots were plated. Samples aliquots were cultured on Modified Oxford agar media and *L monocytogenes* colonies counted after incubation at 35 °C for 2 days.

Results

Raisin extracts appear to have some effect on preventing or reducing the growth rate of *Listeria monocytogenes*. This effect is directly related to the microbial load initially present on the fresh cut produce and the nature of the product. Some fruits and vegetables have some natural phytochemicals that protect them against microbial infection, as it was seen on carrots (data not shown). Celery offers more resistance to *L. monocytogenes* to proliferate compared to cantaloupe melons. At lower initial bacterial load it was harder to perceive differences in the effect of raisin extracts when compared to the control, figures 1 and 2. However, when the fresh cut produce was inoculated with higher bacteria concentrations the benefit of applying raisin extracts was more evident, as seen in figures 3 and 4. The effect was more dramatic for cantaloupe melons inoculated with an initial load of 1000 colony forming units where there was a prevention of about 60 to 80% bacteria growth by applying raisin extracts compared to the control, figure 4. In this experiment, both Thompson (sun-dried) and Golden raisin extracts show very similar effects suggesting they might have compounds in common acting as anti-microbial agents. More experiments should be performed to completely prove this fact.

We also believe that larger antimicrobial effects would be seen if we could obtain extracts with higher amounts of active compounds. Unfortunately, full strength extracts are the highest concentration that can actually be obtained from whole raisins in our

assays. We propose that if raisins are processed in such way that we could obtain a raisin powder (e.g., freeze-dried, spray dried or drum dried), then this product could be re-diluted to the appropriate concentration of active compounds needed for the intended use.

This proposal opens the possibility of creating new products from raisins by controlling the amount of active compounds present. Such approach will allow exploiting the antioxidant and antimicrobial properties of raisins and target markets like Conventional Foods, Functional Foods, Nutraceuticals and Dietary Supplements.

Celery	Mean			Stdev		
	Control	Sundried	Golden	Control	Sundried	Golden
Initial	2.0	2.0	3.0	1.0	1.0	1.0
8 days	0.0	0.3	0.0	0.0	0.6	0.0

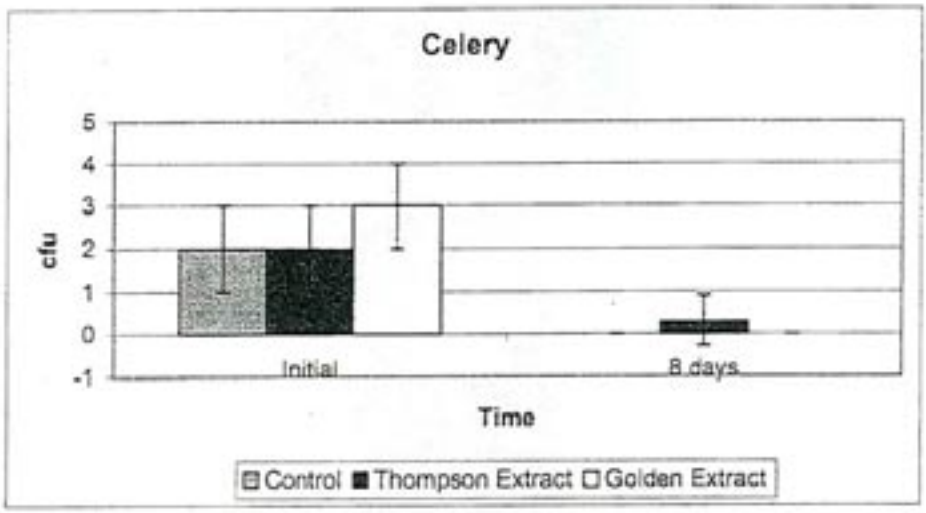


Fig.1. Colony forming units on celery sticks at initial time and after 8 days of storage at 5 °C innoculated with an initial microbial load of 10 cfu.

Cantalope	Mean			Stdev		
	Control	Sundried	Golden	Control	Sundried	Golden
Initial	13.3	14.3	11.7	1.2	4.0	1.2
8 days	219.0	233.0	171.7	29.7	99.9	92.1

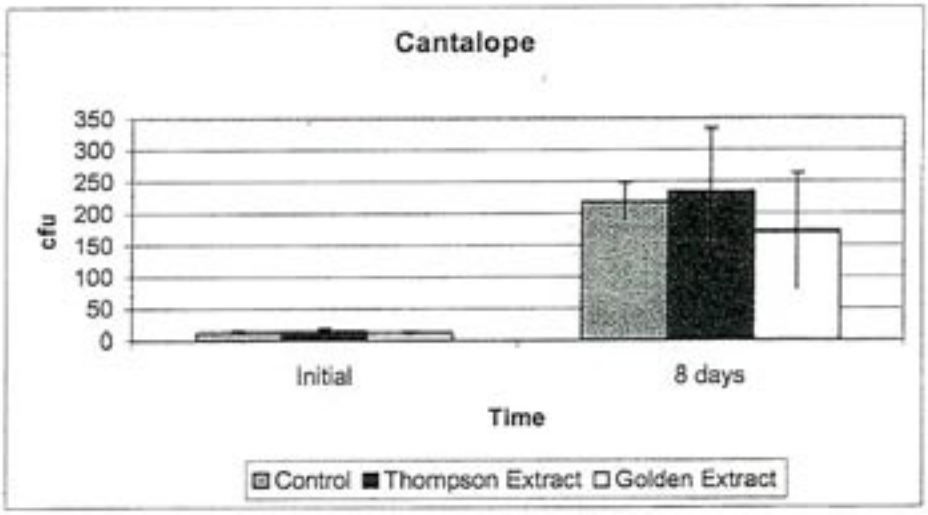


Fig.2. Colony forming units on cantaloupe melon disks at initial time and after 8 days of storage at 5 °C innoculated with an initial microbial load of 10 cfu.

Celery	Mean			Stdev		
	Control	Sundried	Golden	Control	Sundried	Golden
Initial	366.3	482.7	498.3	137.6	51.8	95.0
8 days	550.0	300.0	130.0	428.0	250.0	10.0

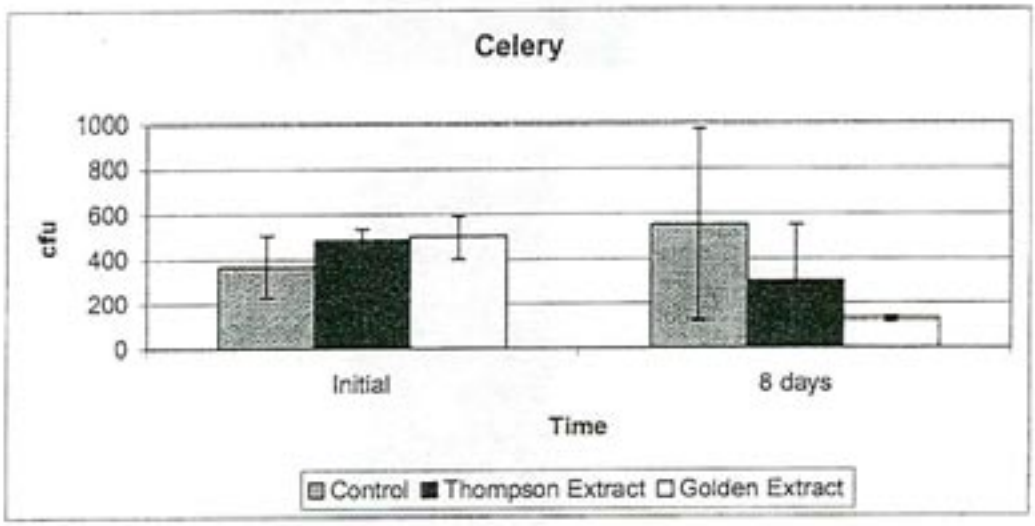


Fig.3. Colony forming units on celery sticks at initial time and after 8 days of storage at 5 °C innoculated with an initial microbial load of 1000 cfu.

Cantalope	Mean			Stdev		
	Control	Sundried	Golden	Control	Sundried	Golden
Initial	995.3	481.0	927.3	85.1	93.0	100.2
8 days	100000.0	19619.0	25993.0	0.0	16457.0	7645.0

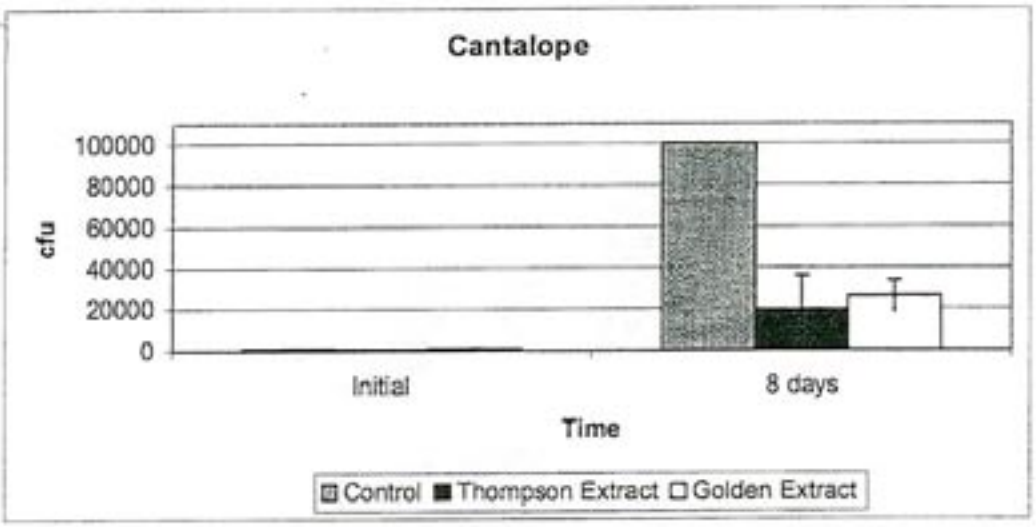


Fig.4. Colony forming units on cantaloupe melon disks at initial time and after 8 days of storage at 5 °C innoculated with an initial microbial load of 1000 cfu.

Abstract presented to the IFT 2001 meeting in New Orleans , June 22-27.

Analysis of raisin extracts to determine phenolic content and antimicrobial activity

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Phenolic compounds are becoming of interest as researchers are discovering functional activities as drugs, colorants, flavors, and antioxidants. Some phenolics share some biological and chemical properties that might be effective inhibitors of chemical mutagens and/or carcinogenesis. Previous studies have shown that naturally occurring hydroxycinnamate derivatives have antimicrobial activity. Phenolic content is also closely associated with the sensory and nutritional quality of fresh and processed plant foods.

Experiments were conducted to determine the type and amount of phenolics present in raisins and test their extracts to identify possible antimicrobial activity.

Total phenolic content, anthocyanins, and antioxidant activity were assayed spectrophotometrically using standardized methods reported in the literature. HPLC was used to separate, identify and quantify the phenolic content. A spectrophotometric test and the Kirby-Bauer Method were used to study the effect of raisins extracts on microbial growth.

Raisins seem to have a considerable content of phenolic compounds. Golden raisins showed the highest concentrations, which can be due to the prevention of browning by SO2 application. Anthocyanins were not detected in any of the raisins analyzed. Golden raisins also showed three times higher antioxidant activity than Thompson-seedless and Zante raisins. Tentative identification of the phenolic profile by HPLC suggests the presence of quinic and gallic acid, chlorogenic and caffeic acids, catechin, epicatechin, and others yet to be identified. Water and methanol extracts from raisins greatly inhibited the growth of L. monocytogenes and E. coli 0157:H7 in the ranges of 70-95% and 50-70% respectively. This results where comparative to a control and 2% chlorogenic acid solution.

Our results suggest that raisins have antimicrobial properties that are related to their phenolic content. Even though much of the phenolics is lost because of browning reactions, the drying process when making raisins concentrates the remaining amounts and make them significant on per weight basis.