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California Raisin Marketing Board 3445 North First Street Suite 101 Fresno, CA 93726 Jan. 1, 2002

Happy New Year

Please find attached the final report for our CRMB sponsored Project "Evaluation of the potential antimicrobial properties of raisins in food safety and Preservation" (Principal Investigator: Dr. M.A. Daeschel, Oregon State University). The report consists of the following in order; project summary, research conducted since the last report, recommendations for future work, periodic research update reports, and the original proposal. We appreciate the support of the CRMB and their patience in allowing us to continue the project beyond the original 1 year time frame.

Sincerely

Mark A. Daeschel

Professor

Project Summary

California Thompson seedless raisins were evaluated for their antimicrobial activity against food spoilage and pathogenic microorganisms. Evaluation was conducted using various raisin extracts in agar diffusion assays. Extracts prepared using 95% ethanol as the extractant were observed to have the greatest antimicrobial activity. Phenolic analysis of the ethanol extract indicated that it had the highest concentrations of phenols as compared to other extracts. Individual phenolic compounds known to be present in raisins were tested for their individual antimicrobial activities. Some activity was observable but was rather limited and only active against specific microorganisms. A beef jerky model food system was developed to evaluate the antimicrobial activity of raisin extracts when applied to the jerky surface. Immersion or spray application of raisin extracts onto the surface of jerky was unsuccessful because of the resultant hygroscopic (sticky) surfaces. Raisins were directly incorporated into jerky formulations at various concentrations and then challenged with food borne pathogenic bacteria. The inclusion of either 25 or 50% raisins in the beef jerky formulation had the effect of reducing the populations of all 3 pathogens as compared to the formulation without raisins. Jerkies containing increasing amounts of raisins also had decreasingly lower pH and water activity values. Based on our data we believe the antimicrobial effects of raisins do not derive from a single component but are the cumulative effect of several chemical and physical factors. Although not exhaustively evaluated, we believe the primary factors are lowered water activity and pH with acidity and phenolic components as secondary contributing factors. Raisins may have several applications as an ingredient in processed meats. We speculate that may be able to substitute for nitrites in regard to antimicrobial and antioxidant functions. Furthermore, they may be a tool to regulate texture, mouth feel, and color in these products. Lastly, they may provide for a more healthy product by virtue of their antioxidant properties and in reducing the fat content by their substitution into processed meats.

Objective:

Previous experiments indicated that surface application of raisin extracts onto beef jerky resulted in sticky surfaces that were impractical for further evaluation. Therefore, raisins will be formulated into beef jerky at 25% and 50%, and then the raisin-jerky will undergo challenge studies using three pathogenic bacteria to evaluate its antimicrobial properties.

Materials and Methods:

Bacteria

Staphylococcus aureus (FFL #B-32) Escherichia coli O157:H7 (ATCC #43895) Listeria monocytogenes (FFL #B-68)

Preparation of Jerky

- 10% lean ground beef
- California Thompson Seedless Raisins
- Smokehouse Jerky Seas flavoring (Eldon Products, Kooskia, ID)
- Curing salt mix (salt, sodium nitrite, dextrose, F,D,& C Red #3)

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0% Raisin Jerky: (100% beef, plus flavorings, curing salts, and water) 25% Raisin Jerky: (75% beef, plus flavorings, curing salts, and water) 50% Raisin Jerky: (50% beef, plus flavorings, curing salts, and water)
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Ingredients were mixed well, and then each formulation was transferred to the Jerky Master (Hi Mountain Jerky, Inc., Riverton, WY) for extrusion. Jerky strips were baked in a convection oven at 200°C for 90 minutes, and then allowed to cool for 30-minutes before bagging. All jerky samples were refrigerated at 4°C until use. Water activity measurements were performed with a Rotronic Hygroskop DT water activity chamber and meter.

Microbial Evaluation of Jerky

Each jerky formulation (0%, 25%, or 50% raisins) was individually challenged with each of three pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*) at a concentration of 10⁴ cfu/ml. Inoculated samples were allowed to incubate at room temperature (25°C). After 48 hrs, jerky samples were placed into stomacher bags with 50 mls of water and homogenized at 260 rpm for 2 minutes (Seward Stomacher 400 Circulator). The resulting sample was then serially diluted and plated for bacterial growth on selective media. Data presented are the mean values from duplicate samples.

Results:

Water Activity

Low moisture formulation (Pepperstix flavoring)

 0% Raisins
 0.888

 25% Raisins
 0.734

 50% Raisins
 0.658

High moisture formulation (Smokehouse flavoring)

 0% Raisins
 0.914

 25% Raisins
 0.720

 50% Raisins
 0.685

(This formulation was used for the microbial expts.)

Antimicrobial Activity

Staphylococcus aureus, Escherichia coli O157:H7, and Listeria monocytogenes are all food borne pathogens that have been implicated in outbreaks associated with the consumption of meat products. As seen with the data given in figure 1., the inclusion of either 25 or 50% raisins in the beef jerky formulation had the effect of reducing the populations of all 3 pathogens as compared to the formulation without raisins. Most dramatic was the effect on Staphylococcus aureus where the microorganism was able to grow on jerky not containing raisins but was killed with the inclusion of raisins. The least affected was E.coli, but still there was increased inhibition in the presence of raisins. The question arises as to what are the components of raisins that contribute to an antimicrobial effect against these pathogens? . At the time we wrote the proposal we were of the mind that it is likely a combinations of several effects working in concert i.e., the hurdle effect. Our experiments with individual phenolic compounds were inconclusive with weak observed antimicrobial activities. Other factors considered are low pH, high acid and osmotic pressure. One factor we did not really consider was the effect of lowered water activity that raisins may impart due to their sugar content. This has become more relevant as we started the current experiments. Looking at the water activity values given above we see that the inclusion of 25 % or 50% raisins gave values below 0.860 which is considered the value at which bacteria of public health concern cannot grow. However, this does not necessarily preclude them from surviving at lower values.

To look at this more closely we formulated beef jerkies (without seasonings and salt) containing 0, 5, 10,15,20,25,30,35,40,45,and 50 % raisin paste, We then determined the water activity and pH values of the finished jerkies. These are given in figs 2 and 3. The 100 % beef jerky had a water activity value of 0.934. The water activity of the raisin containing jerkies reached a value of .860 at the point where the raisin concentration was 20% Concurrently as the water activity decreases we also get a decrease in pH.

Based on our data we believe the antimicrobial effects of raisins do not derive from a single component but are the cumulative effect of several chemical and physical factors.

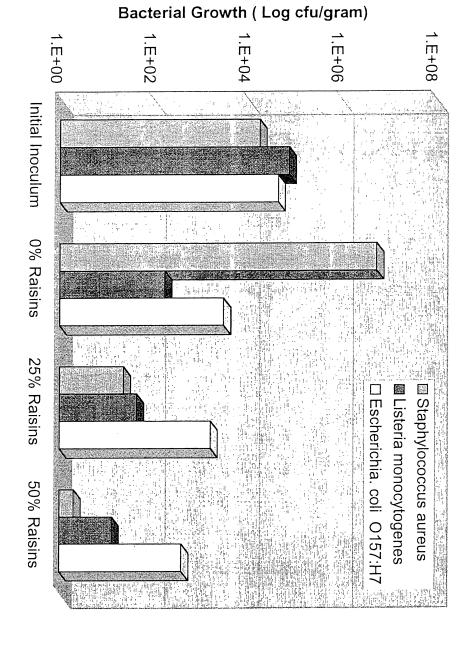
Although not exhaustively evaluated, we believe the primary factors are lowered water activity and pH with acidity and phenolic componets as secondary contributing factors.

Potential Applications and Future Work

To commercialize the antimicrobial properties of raisins in food formulation applications studies would need to be conducted primarily in the area of sensory analysis and nutritive value. These type of studies were not within the scope of our proposal, however we have made several observations in this regard that we wish to share.

- 1) We believe the sensory characteristics of raisins are compatible/complementary to those of specialty processed meat products. These include jerky , pepperoni, salami and meat sticks. We have informally tasted some of our jerky formulations and we generally prefer those with at least $10\,\%$ raisin. Jerky can be tough and chewy. We observed that the inclusion of a small amount of raisin greatly improved the texture and mouthfeel, However the texture began to deteriorate at concentrations greater than 25%
- 2) There is enormous interest in identifying effective substitutes for sodium nitrite use in processed meats. Nitrite functions as an antimicrobial, retards rancidity, and stabilizes color. We believe raisins may value in this regard not only because of their antimicrobial activity but also because of their antioxidant activity which may protect meat products from rancidity. Through our own observations we feel that the color contributions from raisins are completely compatible with processed meat products. In fact raisins could be used to adjust the color of certain products.
- 3) It is obvious that the antioxidant capacity of raisins is important when evaluatingor promoting the health/nutritive value of any processed food that may contain raisins. It should also be obvious that a jerky containing 25% raisin also contains 25% less fat! Fat is important for mouthfeel. However, our observations with tasting raisin formulated jerky was that the mouth feel was actually better than the 100% beef jerky.





pH values of beef jerky formulated with raisins

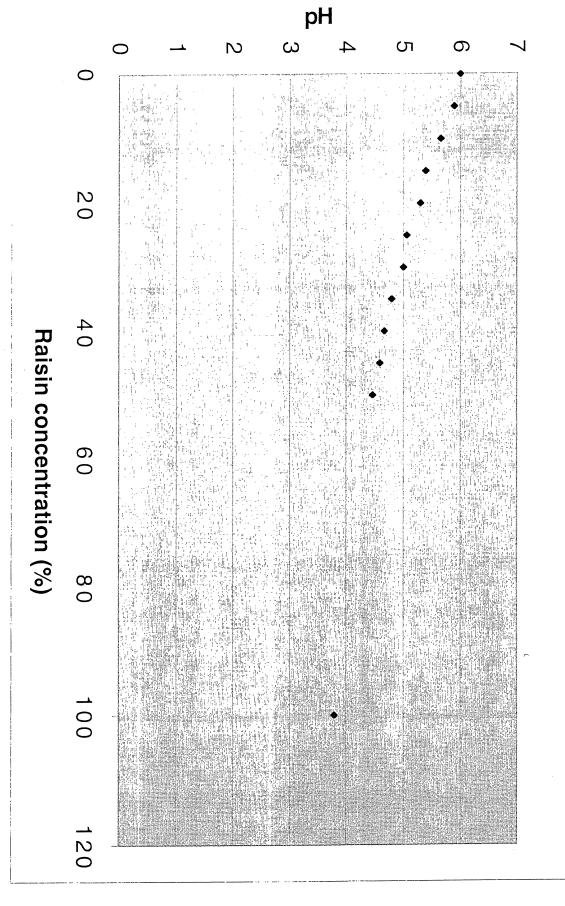
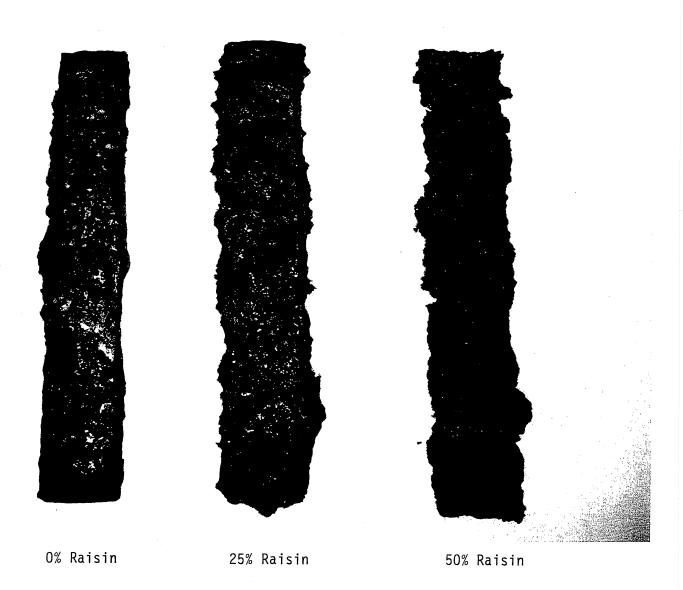


Figure 3.



Raisin formulated beef jerky

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