Department of Agriculture, Trade and Consumer Protection
Division of Agriculture Development
Agricultural Development & Diversification Program (ADD)
Grant Project Final Report

Contract Number: 18032

Project Title: Production, Marketing and Distribution of Carbonated Juice Beverage Product Using Locally Produced Fruit

Project Beginning Date: July 1, 2003 **Projected End Date:** August 31, 2004

Amount of Funding Awarded: $15,000

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1. What were you trying to accomplish with this grant?
The goal of this project was to have the White Winter Winery produce a nonalcoholic "sparkling" blueberry juice without preservatives utilizing local produce that is presently underutilized and undervalued. The grant was designed to: 1) evaluate processing technologies and implement an appropriate production technique to reduce costs to an economically feasible level, 2) determine if a viable market exists for such a product through a feasibility study conducted by "Business Plans, Etc.", 3) provide staff with training to adequately produce the product, 4) conduct research into appropriate technologies for larger scale cost effective production, and 5) provide a framework upon which to build a larger funding request from investors.

2. What steps did you take to reach your goal?
The project started by contacting the University or Wisconsin food science program to set up testing of raw product for pathogen destruction. This testing was conducted by Extension scientist Steve Ingham (see accompanying report). The product was contaminated with three of the most common pathogens; Listeria monocytogenes, Escherichia coli (E.coli), and Salmonella. The hope was the low ph of the product would provide an unfavorable environment for the pathogens, thus allowing no use of preservatives. This phase of the project took longer then expected to implement due to time constraints at the University. When a window did open up for testing, samples were sent, unfiltered, prior to testing. Results indicated pathogen reduction in salmonella and Listeria to acceptable levels but not for E. coli. In addition the samples contained a large amount of yeast which was not affected by low ph levels. In summary, while pathogen reduction was achieved, it did not meet the 5-log reduction required under state law for E. coli. This and the large amounts of yeast were a contributing factor to lack of shelf stability in the product. Telephone conversations with Steve Ingham indicated addition of sulfites would also not ensure a large enough reduction in E.coli as demonstrated in past studies. The conclusion of the UW testing was:

"In order to meet the FDA pathogen reduction standard and achieve shelf stability, thermal pasteurization, or perhaps a multi-stage filtration to remove pulp and microbes is the best option available."

It would have been interesting to do more testing with filtration after contamination, but time, budget constraints, and the fact that the filter could not be re-used after contamination, prohibited this follow-up action. Our present filtration regimen provides sterile filtration at the .45 micron level as standard practice.

One of the positives in the project was information which was gleaned has allowed us to make, at least on a small scale, the Blue Spritz for sale with filtration and the addition of potassium metabisulfite and potassium sorbate. We will need to clarify with Dr. Ingham if the addition of sulfites and sorbate is necessary if sterile filtration is used. It may just be cheap insurance to prevent and ensure that bacterial or yeast growth is inhibited at the time of bottling.

The second major point of the project was to look at market viability. This study was conducted by Business Plans, Etc. and was found to be a defensible project. The study was extensive and will need to be digested over time and put into place. The most effective plan would probably be

to implement in stages at this time. The study indicates equipment can be purchased and run at as little as 36% capacity and still be profitable. However the equipment will take a substantial amount of investment capital, about $350,000, with approximately ¼ to ½ in equity funding. Equity funding is money which comes from sources other then traditional bank financing. This type of funding will usually allow for more liberal pay back terms. Our best possibility for partners in this venture is the growers who will benefit from the fruit we would buy for production. These partnerships have several advantages. First, the growers have a vested interest in our success and thus could provide a more favorable price point for raw ingredients. Second, it would keep more of the dollars in the community and available for re-investment and economic growth and wealth production at the local level. Thirdly, this arrangement could benefit the growers and producer by qualifying for "value-added" marketing, and production grants or loans which the winery cannot presently access despite our emphasis on using locally grown produce.

The third goal was training for staff. The primary focus was for "Hazard Analysis and Critical Control Point" or HACCP training. This training looks at product ingredients and processes from orchard to processing, bottling and storage, to identify possible problem areas and how to correct them before problems occur. Unfortunately this was not able to be accomplished due to the lack of classes held early enough in the year. The winery will pay for this training for staff regardless. The training is presently scheduled for September of 2004.

The fourth goal was to look into equipment for large scale production. Several pieces of machinery were researched for processing, bottling and pasteurizing. We discussed, with reps, the possibility of both heat and pressure pasteurization which is done after bottling. The machinery will need to be evaluated further once financing is available for purchase. The research is in the feasibility study and was completed by Business Plans, Etc... and by Jon Hamilton.

The fifth goal, of building a structure for presentation was accomplished by virtue of the previous four. More work will need to be done to fine tune the presentation. This will allow the presentation to be legally defensible by state law for solicitation of funds and a succinct and fully informed document to present to possible investors and bank finance officers.

3. What were you able to accomplish?

As a result of the project we were able to make a great product with fantastic consumer acceptance. We were able to lay to rest the issue of just exactly how to make the product without preservatives and how to make it immediately with out causing difficulty to consumers. We will be able to refine our processing for the spritz project and, as a by product, for our mead making facility as well through the HACCP training. This will be done after the project ends but the project certainly did provide the impetus for this to occur. We were also able to print labels for a 200 gallon run this summer and announce a new release for the summer season.

4. What challenges did you face?

The primary challenge was faced by time constraints. We were in the middle of a move at the time of the grant project. This meant time was split between many major projects. The project was still completed and completed successfully but could have used more attention. In addition,

the one year grant period was too short. Originally the grant period was for two years to accommodate more extensive testing and evaluation of equipment as well as more effective methods of production. We would have liked to tour other facilities that were using the technology we were looking at to see it in operation and discuss with them how the equipment worked, challenges, etc.. We also were not able to ultimately answer the question of whether or not filtration alone would be adequate to meet the 5-log reduction in pathogens primarily due to time and expense factors. We would have had to destroy a $3000 filter cartridge to answer this question and replace it with another cartridge at the end of testing.

5. What do you plan to do as a result of this project?
We plan to move forward with finding financing for the project and implementing production. Machinery will continue to be evaluated and purchased as we are able to afford it and increase production from year-to-year. We have already used the results to begin production on a small scale. Thus far sales have been brisk out the front door. Further sales will focus on developing outlets in our local market area.

6. How should the agriculture industry or the State of Wisconsin use the results from your grant project?
I believe the results should be used to encourage other entrepreneurial projects in the state involving Wisconsin agriculture. Much of the information which was collected is proprietary to our project, such as recipes, which were developed prior to the project, and the feasibility study, which has specific information on R&D, recipe development, and implementation. As such this information should be treated as confidential. However, the concept of the project can be used to encourage further research and development on similar projects using Wisconsin agricultural products.

Additional Comments
The survey was difficult to answer because our project was geared more to basic product development and market research. As such, many of the numbers are projections for the next year based on production/sales of 70 cases per month. We do estimate to add 1-2 employees at this minimal level of production for distribution, sales and production. We could easily double this number if sales follow the trend established at our show room this summer. The value added to blueberries is an interesting number to consider. Berries are purchased at $1.35/lb and are increased to $28.50/lb by virtue of our process. Our production costs really are not at a point where they will decrease, only increase for awhile yet! We have begun to open new markets with this product already. The primary market focus will be within a 75 mile radius of Iron River to start. This will allow us to self-distribute within this area. We will be able to refine our processing, marketing and distribution while building market close to home.

Investigation of an Alternative to Pasteurization for Pathogen Reduction in Blueberry and Raspberry Juices

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Overview: A Wisconsin processor was evaluating the feasibility of producing and marketing shelf-stable carbonated beverages containing blueberry and raspberry juices. The processor hoped to obtain the FDA-mandated 5-log reduction in pathogenic bacteria using techniques other than thermal pasteurization or ultraviolet irradiation. Alternative treatment options considered were filtration, and a multi-step warm-hold (35^0C, 6h) + freeze/thaw treatment. This study was done to investigate the latter option, as preliminary trials showed that filtration to remove microorganisms would be far too slow for commercial application. The multi-step treatment generally achieved a 5-log reduction of *Salmonella* and *Listeria monocytogenes* in both blueberry and raspberry juice, probably because of the low pH of these juices (pH 2.8 - 3.0). Only a 1.9 - 3.2 log reduction in *Escherichia coli* O157:H7 was achieved, however. The multi-step treatment did not reduce yeast and mold populations sufficiently to allow room temperature storage. It appears that thermal pasteurization, or perhaps a multi-stage filtration to remove pulp and then microbes, is the best option available to attain mandatory pathogen reduction and shelf-stability.

Background

Following several noteworthy outbreaks of food-borne illness linked to contaminated juices, the United States Food & Drug Administration (FDA) enacted regulations requiring juice processors to implement the Hazard Analysis Critical Control Point (HACCP) system and to treat juice such that populations of the target pathogenic microorganism(s) would be reduced 100,000-fold, i.e. undergo a 5-log reduction in numbers (FDA, 2001). Processing techniques and parameters used to achieve the 5-log reduction were not explicitly stated in the regulations. Scientific validation of pathogen reduction techniques, therefore, was to be provided by the processors. The present study dealt with blueberry and raspberry juices, which would fall under the aforementioned FDA regulations. The choice of appropriate target pathogen for these juices was not obvious. There have been no outbreaks of microbial food-borne illness linked to blueberry or raspberry juices. Because windfall fruit is not used, contamination of these berries with *E. coli* Ol57:H7 or *Salmonella* spp. via wild animal feces or manure fertilizer on the ground is unlikely. However, contamination of berries with *E. coli* O157:H7 or *Salmonella* spp via bird feces is quite possible (Refsum et al., 2002; Wallace et al., 1997). It is also possible that berries could become contaminated with *L. monocytogenes* during processing because this pathogen readily colonizes moist niches in food processing plants (Swaminathan, 2001). Therefore, *E. coli* 0157:H7, *Salmonella* spp., and *Listeria monocytogenes* were all tested as target pathogens.

At present, the juice industry primarily uses thermal pasteurization or ultraviolet light treatment to achieve the mandated 5-log reduction in target pathogens. A processing treatment resulting in a 5-log reduction in target pathogens may not necessarily result in a shelf-stable product. Juices treated with typical High-Temperature Short-Time pasteurizers or ultraviolet light units are commonly refrigerated for distribution and sale. Commercial thermal pasteurization treatments used to produce shelf-stable juices are orders of magnitude more severe than thermal pasteurization used with juices that must be refrigerated after pasteurization (Mazzotta, 2001). In the present study, we investigated a multi-step low-technology alternative for achieving pathogen reduction. The multi-step treatment, consisting of a warm-hold (35^0C for 6 h) followed by freezing and thawing ofthejuice had previously been found to achieve a 3 to 5-log reduction of E. *coli* 0157:H7 in apple cider (Ingham and Schoeller, 2002), a juice that is considerably less acidic than the blueberry and raspberry juices studied here.

MATERIALS AND METHODS

Blueberry and Raspberry Juices

Juices were obtained frozen after the 2003 growing season and stored at -20^0C. The pH of the blueberry juice ranged from 2.8 to 2.9 and the pH of the raspberry juice was 2.9-3.0. The sugar content of each juice was evaluated using a temperature-compensated hand-held refractometer (Leica, Inc., Buffalo, NY) and determined to be 10.0-10.2 Brix for the blueberry juice and 11.0 -13.5^0 Brix for the raspberry juice.

Bacterial Strains and Culture Conditions

Three separate cocktails consisting of either five strains of *Escherichia coli* 0157:H7, five strains of *Salmonella* spp., or five strains of *Listeria monocytogenes* were prepared (Table I), and then combined to inoculate juices. Cultures were maintained at -20^0C in Brain Heart Infusion Broth (BHIB; Difco, Becton-Dickinson, Mansfield, MA) containing 10% (w/v) glycerol. Working cultures, maintained at 4^0C on Brain Heart Infusion Agar (BHIA; Difco) plates, were prepared monthly from frozen stock. To obtain working cultures, each strain was cultured twice at 35^0C in BHIB, streaked to a BHIA plate, incubated overnight at 35^0C, observed for culture purity, and then stored for up to 1 month at 5^0C. For each experiment, fresh cultures were prepared by transferring a loop of growth from the working culture plate to 9 ml BHIB and the inoculated tubes were incubated at 35^0C for 20-24 hours. Cocktails were prepared by vortex-mixing each culture, combining the cultures into a 50 ml conical tube (Falcon, Becton-Dickinson, Franklin Lakes, NJ) and centrifuging at 5,000 x g for 12 minutes. The supernatant was decanted and cells were re-suspended to the original volume in the appropriate juice. The three separate cocktails were then

combined and mixed. The inoculated juice was then distributed amongst multiple tubes, with 3.0 ml of inoculated juice added to 30 ml of uninoculated juice in each tube. A tube of freshly inoculated juice was then serially diluted in Butterfield's Phosphate Diluent (BPD; Nelson Jameson, Marshfield, WI) and 0.1 ml of each appropriate dilution was spread-plated on single plates of the appropriate selective medium for initial enumeration of pathogens. Plating media used were Sorbitol MacConkey Agar (SMA; Oxoid, Ogdensburg, NY) for *E. coli* 0157:H7, Xylose Lysine Deoxycholate agar (XLD; Oxoid) for *Salmonella* spp., and *Listeria* Selective Agar with added *Listeria* selective supplements (LSA; Oxoid) for *L. monocytogenes*. The SMA and XLD plates were incubated at 35^0C for 24 h, while the LSA plates were incubated for 48 h at the same temperature. Sorbitol-negative, pale colonies on SMA were counted as presumptive *E. coli* 0157:H7; round, glossy black colonies or red colonies with black centers on XLD were counted as presumptive *Salmonella* spp.; and small-to-medium, brown-to-black colonies surrounded by a black precipitate zone on LSA were counted as presumptive *L. monocyto genes.* In addition, freshly inoculated juices were analyzed for numbers of yeasts and molds using the PetrifilmTM Yeast and Mold Count plates (3M Microbiology Products, St. Paul, MN) and following the manufacturer's instructions.

Treatment of Juices

Each tube of inoculated juice was subjected to one of the following treatments: 1) 6h in a 35^0C water bath (warm-hold) = WH, 2) 24 h at -20^0C, and 24 h at 5^0C (freeze/thaw) = FT, 3) WH + FT, 4) WH + FT + l d storage at 21^0C, 5) WH + FT + 7 d storage at 21^0C, or WH + FT + 14 d storage at 21^0C. Microbiological analyses, as described above, were done following each treatment. Initial trials were done using juices filtered through a 0.45 μM pore-size filter, but filtration was too slow to be practical.

Data Analysis

Three separate trials were done with each juice to evaluate each of the seven treatments described above. A mean value for log CFU (colony-forming unit) per ml of juice was calculated for each treatment. When no colonies were detected a value of the Detection Limit (in log CFU/ml) minus 0.1 was assigned.

RESULTS AND DISCUSSION

Several trends are readily apparent from the experimental results shown in Table 2. Of the three target pathogens tested, *E. coli* Ol 57:H7 was clearly the most difficult to eliminate in blueberry and raspberry juices. These results are consistent with the findings of Roering et al. (1999) and Uljas and Ingham (1999), and reflect the relatively high acid-tolerance of E. *coli* 0157:H7 and the relatively low acid-

tolerance of *Salmonella* spp. and *L. monocytogenes.* Therefore, development of a low-heat treatment that adequately eliminates *E coli* 0157:H7 would provide more than adequate assurance that *Salmonella* spp. and *L. monocytogenes* would be eliminated also. The combination of the warm hold and freeze/thaw steps provided a much greater degree of process lethality than either warm-hold or freeze/thaw alone. Again, these results were consistent with those reported by Uljas and Ingham (1999) for apple cider. All three pathogens survived the multi-step WH + FT treatment better in the raspberry juice than in the blueberry juice. Since the pH values of the two juices were very similar, the poorer pathogen survival in blueberry juice may have been caused by higher levels of phenolic acids or other compounds.

Of most interest is the finding that the multi-step WH + FT treatment generally achieved a 5-log reduction of *Salmonella* spp. and *L. monocytogenes* in either juice, but only caused a 1.9-3.2 log reduction in numbers of *E. coli* 0157:H7. Although further reductions in pathogen numbers occurred during subsequent room-temperature storage, these reductions were probably due to ethanol production as yeasts and molds grew enough to cause spoilage.

In summary, the multi-step WH + FT treatment would not meet the FDA's 5-log reduction requirement if E. *coli* 0157:H7 was considered the target pathogen in blueberry and raspberry juices, but it would be considered effective if either *Salmonella* spp. or *L. monocytogenes* were considered the target pathogen. The multi-step treatment had little effect on yeast and mold numbers, and thus would probably have little effect on refrigerated shelf life of either juice. Use of this treatment would definitely not allow room temperature distribution or storage of either juice. In order to meet the FDA pathogen reduction standard and achieve shelf-stability, thermal pasteurization or perhaps a multi-stage filtration to remove pulp and microbes, is the best option available.

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Table 1. Microorganisms used in evaluating thermal and ultraviolet light pasteurization of grape juice.

Microorganism	Strain Designation	Source
Escherichia coli 0157:H7	ATCC 43895	(1); Ground beef in outbreak
	C7927	(1); Clinical - linked to apple cider
	USDA-FSIS-380-94	(1); Salami implicated in outbreak
	ATCC 51657	(1); Clinical
	ATCC 51658	(1); Clinical
Salmonella enterica serovar Hartford	CDC0778	(2)
Salmonella enterica serovar Rubislaw	CDC F2833	(2)
Salmonella enterica serovar Gaminara	CDC H0662	(2)
Salmonella typhimurium	E40	(3); chicken ovary
Salmonella typhmurium	S-9	(3); Wisc. Lab. of Hygiene
Listeria monocytogenes	H0222	(4); raw potato
	F8027	(4); celery
	F8369	(4); corn
	V7	(3); raw milk
	LM 101	(3); hard salami

(1) American Type Culture Collection, Manassas, VA

(2) Lorrie Friedrich, Citrus Research & Education Center, Lake Alfred, FL

(3) Dr. Eric Johnson, University of Wisconsin-Madison

(4) Dr. Larry Beuchat, University of Georgia, Griifin, GA

Table 2; Destruction of *Escherichia coli* 0157:H7, *Salmonella* spp., and *Listeria monocytogenes* in blueberry and raspberry juices during 1) 6h in a 350C water bath (warm-hold) = WH, 2) 24 h at -20^{0}C, and 24 h at 5^{0}C (freeze/thaw) = FT, 3) WH + FT, 4) WH + FT + 1 d storage at 21^{0}C, 5) WH + FT + 7 d storage at 21^{0}C, or WH + FT + 14 d storage at 21 ^{0}C. Values are mean of three trials (in log CFU/ml) with standard deviation in parentheses.

E. coli 0157:H7

Treatment	Blueberry Juice	Raspberry Juice
None	7.6(0.1)	7.4(0.1)
WH	6.0 (0.1)	6.6 (0.2)
FT	6.4 (0.3)	6.1(0.2)
WH + FT	4.4(0.4)	5.5(0.4)
WH + FT + 1 d	2.9 (0.7)	5.3 (0.7)
WH + FT + 7 d	0.6 (0.6)	0.9 (1.0)
WH+FT+ 14d	0.6 (0.6)	0.6 (0.6)

Salmonella spp.

Treatment	Blueberry Juice	Raspberry Juice
None	7.5(0.1)	7.6(0.2)
WH	2.3(1.3)	4.9(0.4)
FT	2.1(1.4)	3.8(1.1)
WH+FT	1.1(0.3)	2.1(0.3)
WH + FT + I d	0.6 (0.6)	1.3 (0.4)
WH + FT +7 d	0.6 (0.6)	0.6 (0.6)
WH+FT+ 14d	0.6(0.6)	0.6(0.6)

Listeria monocytogenes

Treatment	Blueberry Juice	Raspberry Juice
None	6.8 (0.6)	7.6 (0.1)
WH	1.8(1.6)	5.7 (0.1)
FT	1.9 (1.3)	4.3 (1.4)
WH + FT	0.6 (0.6)	2.6 (1.5)
WH + FT + ~ d	0.6 (0.6)	2.0 (0.6)
WH + FT +7 d	0.6 (0.6)	0.6 (0.6)
WH+FT+ 14d	0.6(0.6)	0.6(0.6)