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A Holistic View of the Fate of Berry-Derived Adjuncts throughout Fermentation

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Abstract: Berries and their products can enhance the antioxidant profile, color, and sensory characteristics of beverages, resulting in competitive, value-added products. However, a complete overview of how fermentation affects these compounds is lacking. The American black elderberry, *Sambucus canadensis*, is an excellent candidate for studying how berry juices are affected during fermentation due to high concentrations of color compounds, anthocyanins, and volatile compounds. Gravity, pH, titratable acidity, total anthocyanins, color, and GC-MS analyses were performed on two varieties of elderberries to examine the physical and chemical qualities of elderberry juice before and after wine fermentation. A commercial product with elderberry adjuncts added post fermentation was also analyzed. The concentration of anthocyanins degraded by ~40% as the color of the elderberry wine shifted from blue to red after fermentation. Products that added elderberries post fermentation did not see the same degradation, however, also did not incorporate the same changes to volatile compounds as observed in the fermented wine. The fermentation industry can use this study's findings to decide how best to use fruits to improve commercial products.

Keywords: elderberries; fermentation; adjunct; wine; anthocyanins; aroma; volatile compounds; bioactive compounds

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1. Introduction

Berries and berry-derived products can be added before or after fermentation, resulting in different outcomes. Berry adjuncts can enhance a product's color, antioxidant profile, and sensory characteristics [1]. Fermented alcoholic products such as wines, ciders, and beers are often combined with fruits to achieve unique aromas and flavors [2]. Incorporating berry adjuncts before or after fermentation is a critical decision that impacts the chemical, kinetic, and sensory characteristics of fermentation and the final product differently. Berries are a rich source of bioactive compounds such as ascorbic acid and anthocyanins [3]. Incorporating adjuncts derived from berries into fermented beverages results in a unique, value-added product that increases the shelf-life of these normally perishable fruits [1]. Fermentation can also increase the bioavailability of phenolics such as anthocyanins by liberating insoluble bound compounds from the plant matrix [4]. Bioactive and color compounds are broken down and changed through chemical processes during fermentation. Berry adjuncts often contain natural sugars that can be used by yeast for fermentation or to sweeten a product after fermentation. Previous researchers have studied individual aspects of berry adjuncts in fermentation processes, such as anthocyanins, color, and volatile compounds [5,6]. However, a comprehensive look at how berries influence these fermentation parameters has been lacking thus far. With a holistic evaluation of these parameters, brewers, winemakers, and other beverage producers can design better products incorporating changes throughout fermentation.

The American black elderberry, *Sambucus canadensis*, is an excellent candidate for examining how berry juices will be affected during fermentation. Elderberries contain high levels of color compounds, bioactive (particularly anthocyanins), and volatile compounds.

These compounds are common in many fruits, such as strawberries and blueberries. Elderberries contain bioactive compounds such as B vitamins, A vitamins, tocopherols, vitamin C, flavonols, phenolic acids, proanthocyanidins, anthocyanins, and minerals [7]. Anthocyanin compounds, primarily cyanidins, give elderberries their purple-black color [8]. *S. canadensis* contains variable amounts of cyanidin-3-(Z)-(p-coumarin)-sambubioside-5-glucoside, cyanidin-3-(p-coumarin)-glucoside, cyanidin-3-(E)-(p-coumarin)-sambubioside-5-glucoside, and cyanidin-3-(p-coumarin)-sambubioside based on berry variety and growth environment [7]. Bioactive compounds have been shown to degrade during fermentation [5]. Anthocyanins have been identified as potential pharmaceutical ingredients due to their antidiabetic, anticancer, anti-inflammatory, antimicrobial, and antiviral properties [9,10]. Food product developers may find anthocyanins a desirable adjunct to their products as a result.

Many color compounds in fruits are phenolic compounds, of which anthocyanins are a subcategory. The food industry uses fruit adjuncts to achieve desirable color attributes without synthetic colorants. For example, beet juice has been utilized to add a purple-red color to products from brewing to ice cream. Elderberries are black and purple in their raw form [8]. Synthetic colorants face consumer and regulatory scrutiny, making natural, fruit-based colorants desirable. Fruit colorants are associated with unique challenges, such as stability to degradation and environmental influence on color. Color degradation can result from chemical changes during fermentation and is compounded as pH decreases. However, complete color degradation is uncommon [9]. Some fruit-based compounds are more stable to environmental changes than others [11].

The volatile profile of a food or beverage product influences its aroma and flavor. Fruit addition to fermented products can enhance sensory attributes. Various commercial examples of fermented fruit wines, beers, ciders, and kombucha exist. Elderberry volatiles consist primarily of alcohols, aldehydes, and esters. Previous studies have found that the characteristic fruity-sweet, floral elderberry aroma is due to β-damascenone, dihydroedulan, ethyl-9-decenoate, 2-phenyl ethanol, phenylacetaldehyde, nonanal, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, methyl heptanoate, methyl octanoate, and methyl nonanoate [12]. Phenylacetaldehyde (32.3%), benzaldehyde (7.9%), ethyl linoleate (5.4%), 4-vinyl guaiacol (4.9%), linalool (4.5%), and phenyl ethyl alcohol (4.1%) were the most abundant volatile compounds identified in elderberries [13]. Fermentation alters volatile compounds, typically increasing aroma complexity [14]. The strain of yeast used during fermentation impacts which volatile compounds are metabolized. Lower alcohols, organic acids, and terpenes are often consumed to produce higher alcohols and esters in fruit wines [15]. Volatile compounds that contribute to the aroma of elderberries may be degraded below the odor threshold during fermentation. Aroma odor thresholds describe the minimum concentration at which a person can detect a particular aroma compound [15].

This study builds upon the work of previous researchers who studied independent components of berry adjuncts by holistically assessing the fermentation effect on anthocyanin concentration, color, volatiles, and acidity while also incorporating commercial samples. Juice from two elderberry varieties (Variety 1 and Variety 2) was analyzed before and after wine fermentation. Anthocyanins, color, volatile composition, total acidity, and pH were measured. The volatiles were categorized by berry variety and compounds common to elderberries overall. A commercial example of an elderberry cider and its cider base were also analyzed. The elderberry cider was created post-fermentation by adding elderberry juice and was compared to the wines in which elderberry adjuncts were added before fermentation. The cider and wine comparison provides insight into the various methods to incorporate berry adjuncts. This study provides a holistic view of how fruits can be added to fermentation. The beer, wine, and beverage industries can use these insights to decide best how to use adjuncts when designing novel fruit products.

2. Materials and Methods

2.1. Materials

An industry partner donated elderberry juice which encompasses harvests from 2023. Two varieties of elderberries, termed Variety 1 and Variety 2, were utilized in this study. Juice from these varieties was fermented according to winemaking techniques, with samples taken at the beginning and end of fermentation. Additionally, a commercial (apple-based) cider was provided and analyzed before (cider base) and after elderberry juice addition.

2.2. Gravity Measurements

The original and final gravity of the must was measured using the ASBC official method Beer-4-E (instrumental method for Alcohol and Original Gravity) using an Anton Paar (Gesellschaft mit beschränkter Haftung) DMA 35 Standard portable density meter and an ALEX 500 (Alcohol and Extract Meter) [16].

2.3. pH

pH was measured using an Orion pH meter (Waltham, MA, USA). Before use, the pH meter was calibrated using a three-point calibration curve (4, 7, and 10).

2.4. Titratable Acidity

Titratable acidity (TA) was determined by diluting 5 mL of wine with 125 mL of deionized water and then titrating the sample to a final pH of 8.2 with 0.1 N NaOH. Titratable acidity was reported as grams of citric acid per liter of wine [17] or malic acid per liter of wine or cider.

2.5. Total Anthocyanins

Total anthocyanins were measured using the AOAC Official Method 2005.02: Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines [18]. This is a pH differential method.

2.6. Color

Wine color intensity and hue were quantified using the Glories method [19]. The Glories method utilizes a UV–Vis spectrophotometer to measure absorbance at 420, 520, and 620 nm. Adding these absorbance values measures the color present in the wine. Wine hue is measured by dividing the absorbance at 420 nm (yellow) by the absorbance at 520 nm (red), correlating to the aging of wine. To adjust elderberry wine to appropriate absorbance levels, wine samples were diluted 10× before measurements, and a cuvette with a 2 mm pathway was used.

$$Wine\ Color\ Intensity = A_{420} + A_{520} + A_{620} \quad (1)$$

$$Wine\ Hue = A_{420}/A_{520} \quad (2)$$

2.7. Extraction of the Volatile and Semi-Volatile Compounds

Extraction and concentration of the volatile and semi-volatile compounds in the beer were performed using solid-phase microextraction (SPME). A 50/30 um Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/Carboxen/PDMS) fiber (Supelco, Inc., Bellefonte, PA, USA) was exposed to the headspace above 10 mL of sample spiked with 25 µL 2-Octanol (20 mg/L) as the internal standard. The internal standard was made by adding 25 µL 2-Octanol in 50 mL of 200-proof ethanol. The sample also contained 30% *w*/*v* of salt in 20 mL headspace vials with Teflon-lined silicone septa. Samples were equilibrated at 40 °C for five minutes before exposing the fiber (Chromacol, Fisher Scientific, Hampton, NH, USA) for 30 min at 40 °C with an agitation speed of 250 rpm [20].

2.8. Gas Chromatography-Mass Spectrometry (GC-MS)

Volatile compounds were desorbed for five minutes in the injection port of a Shimadzu gas chromatograph (GC) 2010 Plus Series mass spectrometer detector (MSD) QP2010 SE (Columbus, MD, USA). The injection port was set to 250 °C, and all injections were made in splitless mode using a narrow bore, deactivated glass insert. Volatile compounds were separated using a nonpolar ZB-5MS (ZB; 30 m × 0.25 mm id × 0.25 μm film thickness) with helium as the carrier gas at a flow rate of 2.0 mL/min (linear velocity 53.8 cm/s). The GC oven temperature program was set at 35 °C, held for 5 min, and then increased to 225 °C at a rate of 6 °C/min. Once the final temperature of 225 °C was reached, it was maintained for 10 min. The MSD was maintained at 200 °C, and the sample mass was scanned in the range of 40–800 m/z. GC-MS was performed to separate and identify the volatile and semi-volatile compounds in the wine samples. Peaks were identified using standardized retention time (retention index values, RI), pure compounds and fragmentation spectra of standards, and the Wiley 2014 mass spectral library [20]. A commercially available carbon alkane standard (C6–C20; Sigma-Aldrich; St. Louis, MO, USA) was run to obtain LRI values. The alkane standard was run using the same chromatography method previously described.

$$RI = 100\,N + 100\,n\,(t_{Ra} - t_{Rn})/(t_{R(N+n)} - t_{RN}) \quad (3)$$

N is the carbon number of the lowest alkane, and n is the difference between the carbon number of the two n-alkanes that are bracketed between the compound; t_{Ra}, t_{Rn}, and $t_{R(N+n)}$ are the retention times of the unknown compound, the lower alkane, and the upper alkane.

2.9. Statistical Methods

Statistical analyses were conducted using coding for R (R Project for Statistical Programing, Vienna, Austria). Statistical calculations were completed using a one-way Analysis of Variance (ANOVA) with $\alpha = 0.05$).

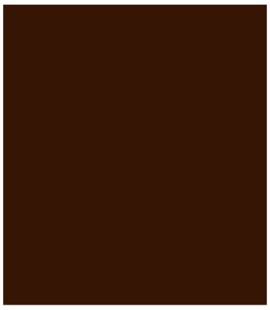
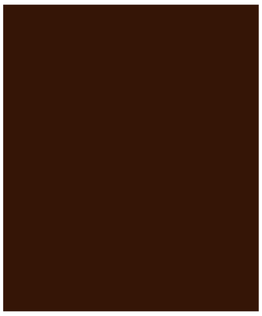
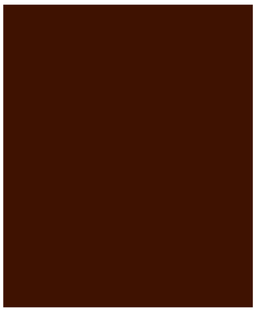
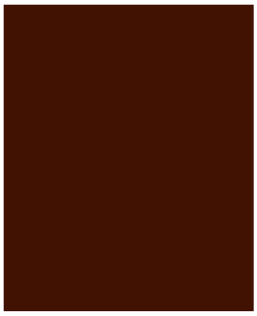
3. Results and Discussion

Differences were observed between the pH, total acidity, solids content, anthocyanins, and color of two elderberry varieties, Variety 1 and Variety 2 (Table 1). These parameters were measured before and after wine fermentation. Fermentation appeared to increase acidity and utilize solids as expected, and anthocyanins were degraded. An increase in red color was observed.

Throughout the ethanolic fermentation, it was observed that the concentration of anthocyanins decreased by approximately 40% in both cases (Table 1). While the initial concentration was higher in elderberry juice, this decrease is comparable to values found by Hornedo-Ortego et al., 2017, who studied anthocyanin degradation during strawberry wine fermentation [5]. When fermenting with juices high in anthocyanin potential, some degradation during fermentation should be expected, as should residual anthocyanin potential. Elderberry wine production followed similar trends to other wine fermentations, such as decreased pH and °Brix and increased total acidity. Atypically, there was no visible change in color between the elderberry juices and elderberry wines.

Two commercial products, an elderberry cider and its respective cider base, were also analyzed. Adding elderberry juice after cider fermentation increased the total anthocyanin content (Table 2). When commercial samples incorporating elderberry juice at the end of fermentation were assessed, the elderberry juice could be considered an inclusion that did not undergo degradation. However, the juice was diluted by the inclusion ratio. From a physiochemical standpoint, this may seem to be the preferred option, as all the anthocyanins and color compounds are retained in the final product. However, the products will be inherently different as the volatile composition will also change during fermentation, as yeast will utilize a portion of higher alcohols for ester production. Therefore, the consumer will be able to detect differences between juices that have undergone fermentation and those used as an inclusion. Examples of products with elderberry inclusions include vitamin gummies, cough syrups, teas, lemonades, and syrups.

Table 1. Characteristics of two elderberry varieties, Variety 1 and Variety 2, before and after wine fermentation.

Parameter	Variety 1 Juice	Variety 2 Juice	Variety 1 Wine	Variety 2 Wine
pH	4.61 ± 0.01	4.86 ± 0.02	3.97 ± 0.01	3.96 ± 0.01
Total Acidity as Citric Acid (g/L)	6.87 ± 0.30	4.74 ± 0.26	8.53 ± 0.74	8.96 ± 0.10
Solids (°Brix)	11.87 ± 0.10	10.30 ± 0.10	1.87 ± 0.06	2.33 ± 0.10
Total Anthocyanins (mg/L)	3931.1 ± 60.5	6765.8 ± 334.6	2288.8 ± 531.3	4005.4 ± 38.8
Color (Red%)	50.72 ± 1.93	49.61 ± 0.37	56.69 ± 1.42	58.53 ± 0.40
Color Hex Code	331C0E	321C0F	391909	3B190A
Color				

N = 3; Mean ± SD. Note, for each variety, there were significant differences in all parameters between the respective juice and wine.

Table 2. Physical characteristics of a commercial cider base product and an elderberry cider made from the cider base with an elderberry juice addition.

Parameter	Commercial Cider Base	Commercial Elderberry Cider
pH	4.12 ± 0.01	4.07 ± 0.01
Total Acidity as Malic Acid (g/L)	3.80 ± 0.39	3.80 ± 0.39
Solids (°Brix)	0.3 ± 0.1	0.57 ± 0.1
Total Anthocyanins (mg/L)	0 ± 0	355.7 ± 114.4
Color (Red%)	32.65 ± 0.48	37.11 ± 0.84

N = 3; Mean ± SD.

GC-MS analyses revealed changes in volatile compounds from elderberry juice to elderberry wine (Table 3).

The volatile compositions of the elderberry juices and wines were diverse, resulting in changes in the aroma profile from juice to wine (as expected). Some components increased, others decreased, and other volatile compounds became different compounds through chemical synthesis. Isoamyl acetate is present in both the original juices and wines. However, in both varieties, there is an increase in isoamyl acetate after fermentation. This represents how yeast can produce isoamyl acetate, although the concentration is affected by yeast species, stress, and temperature. Phenylethyl alcohol decreased as it was partially converted to phenylethyl acetate during fermentation. A corresponding change in flavors associated with this compound (whiskey, floral, fruity, and sweet) was expected after fermentation. Alcohols and esters comprise the majority (by concentration) of volatile compounds identified.

Volatile compounds were also identified in the two cider products to observe the impact of elderberry additives on beverages (Table 4).

Table 3. Volatile compounds identified in elderberry juices and elderberry wines.

Compound	Odor Descriptors	Odor Threshold (µg/L)	Concentration (µg/L) Variety 1 Juice **	Variety 2 Juice *	Variety 1 Wine *	Variety 2 Wine *
Alcohols						
1-Octen-3-ol	Mushroom [21]	0.1 [21]	314 ± 544	679 ± 204	29.1 ± 0.05	-
Benzyl alcohol	Almonds, bitter [22]	5500 [23]	835 ± 1099	524 ± 126	58.2 ± 28.0	54.2 ± 67.5
Isoamyl alcohol	Whiskey, fusel oil [24]	42 [25]	-	2210 ± 1448	22,492 ± 3128	4956 ± 1491
2-Decanol	Coconut, aniseed [22]	0.77 [26]	9.09 ± 7.89	-	5.21 ± 9.03	3.70 ± 6.41
1-Hexanol	Green, fruity, sweet alcohol [27]	6.0 [26]	-	-	38.3 ± 33.4	40.7 ± 70.5
p-Methoxybenzyl alcohol	-	-	428 ± 742	-	-	-
Phenylethyl alcohol	Floral, rose, dried rose, bready, sweet [24]	140,000 [28]	764 ± 958	15,062 ± 3579	10,188 ± 1895	12,789 ± 2082
SUBTOTAL			3026 ± 289	21,392 ± 2842	35,685 ± 5396	19,410 ± 3155
Benzene						
Styrene	Sweet [29]	35 [26]	32.98 ± 57.12	-	-	315 ± 55.2
SUBTOTAL			32.98 ± 57.12	-	-	315 ± 55.2
Esters						
Isoamyl acetate	Sweet, winy [24]	3.4 [27]	12.3 ± 21.4	95 ± 134	2209 ± 1464	647 ± 126
Ethyl 2-hydroxy-4-methylpentanoate	Fresh blackberry [30]	51 [30]	40.0 ± 46.0	410 ± 285	740 ± 165	192 ± 8.14
Ethyl hexanoate	Fruity, pineapple–banana, winy [24]	154,600 [28]	51.8 ± 89.8	54.4 ± 77.0	1300 ± 516	798 ± 250
Ethyl octanoate	Fruity, floral, winy, apricot [24]	580 [28]	286 ± 323	426 ± 169	8817 ± 1098	4323 ± 587
Ethyl decanoate	Oily, cognac-/brandy-like [24]	200 [30]	476 ± 615	360 ± 128	5690 ± 373	2985 ± 296
Ethyl dodecanoate	Oily, fatty, fruity [28]	1500 [28]	194 ± 261	105 ± 46.7	875 ± 231	517 ± 47.6
SUBTOTAL			2497 ± 181	4081 ± 179	23,394 ± 1621	12,336 ± 846
Terpenes						
Linalool	Floral, fresh [24]	0.0032 [30,31]	1035 ± 451	11,920 ± 5134	691 ± 110	4757 ± 643
SUBTOTAL			1212 ± 321	13,084 ± 3166	756 ± 278	5436 ± 1484
Other						
2,3-Butanediol	Rubber, sweet, warming, diacetyl, butterscotch [22]	1500 [28]	197 ± 180	1010 ± 419	2687 ± 1194	1432 ± 399
m-Di-tert-butylbenzene	-	-	1.45 ± 2.51	-	301 ± 467	11.1 ± 10.0

* N = 3; Mean ± SD; ** N = 2; Mean ± SD. - indicates a compound was not detected. Odor attribute information is from [21–32]. Odor thresholds were measured in water.

Table 4. Volatile compounds identified in a commercial cider product (base) and an elderberry cider made from the cider with elderberry juice addition.

Compound	LRI	Odor Descriptors	Odor Threshold (µg/L)	Concentration (µg/L) Commercial Cider Base *	Commercial Elderberry Cider *
Alcohols					
1-Octen-3-ol	970	Mushroom [16]	0.1 [16]	-	-
Benzyl alcohol	1036	Almonds, bitter [17]	5500 [18]	-	-
Isoamyl alcohol	-	Whiskey, fusel oil [19]	42 [20]	8313 ± 3656	23,690 ± 726
2-Decanol	1207	Coconut, aniseed [17]	0.77 [21]	368 ± 303	26.8 ± 16.7
Phenylethyl alcohol	1115	Floral, rose, dried rose, bready, sweet [19]	140,000 [24]	6086 ± 3102	7112 ± 690
SUBTOTAL				15,703 ± 2225	31,472 ± 5723
Esters					
Isoamyl acetate	882	Sweet, winy [19]	3.4 [27]	5113 ± 2195	7870 ± 739
Ethyl 2-hydroxy-4-methylpentanoate	1057	Fresh blackberry [33]	51 [33]	-	17.0 ± 29.5
Ethyl hexanoate	1003	Fruity, pineapple–banana, winy [19]	154,600 [24]	1728 ± 723	1739 ± 626
Ethyl octanoate	1177	Fruity, floral, winy, apricot [19]	580 [24]	11,697 ± 3100	1191 ± 693
Ethyl decanoate	1385	Oily, cognac-/brandy-like [19]	200 [24]	5208 ± 996	595 ± 207
Ethyl dodecanoate	1514	Oily, fatty, fruity [24]	1500 [24]	163 ± 27.2	26.9 ± 46.5

Table 4. *Cont.*

Compound	LRI	Odor Descriptors	Odor Threshold (µg/L)	Concentration (µg/L)	
				Commercial Cider Base *	Commercial Elderberry Cider *
SUBTOTAL				26,729 ± 2738	13,924 ± 1596
Ketones					
β-Damascenone	1368	Stewed apple, honey, kettle hop, tobacco, coconut [17]	0.001 [17]	391 ± 645	49.9 ± 45.8
SUBTOTAL				428 ± 189	
Terpenes					
Linalool	1097	Floral, fresh [19]	0.0032 [28]	-	48.8 ± 84.5
SUBTOTAL				812 ± 172	125 ± 19.8
Other					
2,3-Butanediol	804	Rubber, sweet, warming, diacetyl, butterscotch [17]	1500 [24]	79.2 ± 117	9.37 ± 16.2
m-Di-tert-butylbenzene	1244	-	-	427 ± 372	118 ± 38.1

* N = 3; Mean ± SD; - indicates a compound was not detected. Odor attribute information is from [16–28,33]. Odor thresholds were measured in water.

During processing, there was an apparent loss of volatiles between the cider base and the elderberry cider. This was probably a by-product of the carbonation process. However, it was evident that adding elderberry juice led to an increase in specific juice-associated volatile alcohols. As the juice was not fermented, there was no overall increase in esters (as expected). This result highlights that the juice addition to the final product will result in a fundamentally different volatile profile and associated flavors than fermented juice. Winemakers, brewers, and beverage producers can use this information to create beverages that are tailored to the specific demands of their consumers' palates.

4. Conclusions

Elderberries contribute color, bioactive, and volatile compounds to fermented beverages when used as adjuncts. Elderberry adjuncts added after fermentation resulted in the preservation of anthocyanin by preventing degradation. Volatile compounds were altered during fermentation, resulting in the loss of some alcohols as they were converted to acetates. In studying a commercial cider base to which elderberry juice was added after fermentation, color, bioactive, and volatile compounds were preserved, but no fermentation specific compounds (e.g., many esters) were formed. The information gleaned in this study on the fate of berry-derived adjuncts in fermentation contributes to a broader understanding of fruit additives (adjuncts) use in beverage production.

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