IMPACT OF PROTEIN AND MALIC ACID ON FOAM CHARACTERISTICS OF SPARKLING WINES MADE FROM GRAPES OTHER THAN TRADITIONAL *VITIS VINIFERA* CULTIVARS

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ABSTRACT

Protein and malic acid are known to have an impact on the foam characteristics of sparkling wine. However, it is unknown to what extent they promote foam height and stability in sparkling wine made from grapes other than traditional *Vitis vinifera* cultivars. The aim of this study was to investigate the influence of proteins (via BCA-200 Protein Assay) and malic acid (via OenoFOSS FTIR) in non-*vinifera* grapes on sparkling wine foam height and time until 50% collapse, determined by Krüss Dynamic Foam Analyzer. Fifteen commercially available sparkling wines, made from native or interspecific hybrids and with different production methods, were sourced from the Finger Lakes region of New York. In the wines studied, the interaction of protein and malic acid concentrations were found to affect foam height and malic acid approached significant effect on foam stability. Contrary to expectations, production method, rather than grape cultivar, showed the greatest influence on foam height and stability. Foam height was favored by forced carbonation, and foam stability was significantly decreased by the transfer method. These findings represent the start of a broader investigation to provide industry with an understanding of the compositional factors and carbonation methods driving foam characteristics of sparkling wines made from non-*vinifera* grapes.

BIOGRAPHICAL SKETCH

Adrienne Lindstrom has a diverse background that has led her on her journey to winemaking. She earned her Bachelor's degree in Theatre and Spanish at SUNY Oswego in upstate New York in 1995. After a brief acting career with a touring children's theatre company, Adrienne accepted a position as a 4th grade Spanish immersion teacher in Philadelphia. During her twelve-year teaching career, she earned her Master's of Education from Eastern University and served as a Fulbright Scholar to Uruguay. While there, Adrienne tasted wines made from the Tannat grape, a grape she had never heard of before. That experience inspired her to start learning more about wine and in 2014 she started volunteering and interning at wineries in the New England area. In 2018, Adrienne began her studies at Cornell University in pursuit of a Master of Professional Studies with a focus on Enology and special emphasis on sparkling wines.

ACKNOWLEDGMENTS

The task of completing this work could only have happened with the help and patience of the many people in the Cornell University Food Science Department.

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I would like to thank all the participating wineries that graciously donated their wine for this study and the New York Wine and Grape foundation for project funding.

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Finally, I owe a special thanks to my husband for his support and confidence. Matt has been my rock through this whole process. Without his love and encouragement, I would not have taken the risks that lead to where I am. Thank you for putting up with the rambling phone calls and long drives.

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INTRODUCTION

Sparkling wine is produced in most wine making regions of the world; because its consumption is often associated with celebrations or special occasions, it commands a unique and high quality space and price in the wine market (Kemp et al. 2018). Sparkling wine differs from still wine by exhibiting a foam on the surface of the wine when poured in a glass, which consumers deem attractive (Cilindre et al. 2010, Kemp et al. 2018). Carbonation is introduced through one of four methods, involving either direct CO_2 infusion into a still base wine (forced carbonation) or generation of CO_2 via secondary fermentation in pressurized tanks ('Charmat' method) or in the bottle (transfer method and Méthode Traditionelle). The progressive release of dissolved CO_2 bubbles to produce foam (Pozo-Bayón et al. 2009) during pouring is the first quality marker of sparkling wine perceived by the consumer (Martínez-Lapuente et al. 2015). The foam eventually effervesces to leave a bubble ring, known as the collar, at the periphery of the wine along the glass. These three characteristics: foam, effervescence, and collar- are greatly valued by the consumer during tasting (Cilindre et al. 2010). While other quality markers like taste and aroma are also important, the initial, visual signals of foam formation and persistence on the surface are critical in the assessment of a quality sparkling wine (Esteruelas et al. 2015, Pozo-Bayón et al. 2009).

Sparkling Wine Foam

Foam quality is defined by a slow release of CO_2 from the depths of the liquid via multiple streams of bubbles rising from different points of the glass. As bubbles rise, they contribute to the formation of a crown of foam of bubbles over the surface of the liquid (Martínez-Lapuente et al. 2015). Most sources agree that the bubbles should be tiny and delicate rather than boisterous and frothy (Liger-Belair et al. 2001). The collar that forms against the sides of the glass should

consist of tiny bubbles lasting anywhere from a few seconds to several minutes. (Cilindre et al. 2010). The stability of the collar is a criterion of quality for the consumer (Brissonnet and Maujean 1993, Vanrell et al. 2007)

Foam Formation and Stabilization

Upon pouring, surface foam develops because the liquid is supersaturated with dissolved CO_2 molecules which must escape through the gas-liquid interface to reach the vapor phase (Liger-Belair et al. 2004). This foam is naturally unstable because the surface tension of the bubbles counteracts the force needed to maintain their shape, ultimately leading to the collapse or coalescence of the foam (Blasco et al. 2011). Consequently, foam formation and stability depend on compounds that decrease the surface tension on the bubble surface and increase the viscosity on the film between bubbles (López-Barajas et al. 1997). In sparkling wines, these compounds are proteins (Martínez-Lapuente et al. 2015)

Proteins are important to foam characteristics because they act as surface active compounds (surfactants) by unfolding and accumulating on the gas-liquid interface of the bubble (Condé et al. 2017a, Esteruelas et al. 2015, Pozo-Bayón et al. 2009).

Proteins bind together and interact with the interface of the bubble by means of hydrophobic forces, hydrogen bonds or covalent linkages (Blasco et al. 2011). These interactions lead to an elastic film that reduces surface tension, enhances foam stability, and seems to contribute to the film's elasticity and strength (Blasco et al. 2011, Vanrell et al. 2007). However, all proteins do not contribute equally to the foaming properties of wines (Esteruelas et al. 2015, Wilson 1989). Brissonet and Maujean (1993) showed that hydrophobic proteins contribute more to bubble and foam characteristics than hydrophilic proteins.

The majority of grape proteins present in sparkling base wines have been identified as thaumatin-like proteins (TLPs) and chitinases (Culbert et al. 2017). TLPs have been reported to promote desirable foam properties by enhancing foam volume and foam stability while chitinases reportedly have no significant effect (Condé et al. 2017a, Esteruelas et al. 2015). Depending on production methods, yeast mannoproteins may also be present in the finished sparkling wine; these are of particular importance to foam stability as their hydrophobic nature causes them to preferentially adsorb to the gas/liquid interface of foam bubbles (Blasco et al. 2011).

Grape-derived proteins are relatively small and have molecular weights between 9.6 and 60 kDa, with the majority falling between 20 and 30 kDa (Brissonnet and Maujean 1993). TLPs have a molecular weight of 24 kDa and yeast-derived mannoprotein molecular weights range from 10 to 200 kDa (Blasco et al. 2011, Esteruelas et al. 2015). Studies of ultra-filtered wines stripped of molecules larger than 3.5 kDa did not produce any measurable foam, confirming the importance of macromolecules to foam formation (Aguié-Béghin et al. 2009). Wines with higher molecular weight yeast mannoproteins had higher foaming ability when compared to wines with grape berry proteins only. The greatest foam height was found when protein fractions were combined suggesting a synergistic interaction between yeast mannoproteins and grape proteins (Kemp et al. 2018, Vincenzi et al. 2014).

While it should be possible to estimate the foam potential of a wine by protein concentration (Kemp et al. 2018, López-Barajas et al. 1997), the only reported attempt to correlation protein analysis and foam height was unsuccessful (Condé et al. 2017). To date, the literature discussing the influence of proteins in sparkling wine foam formation and stability has

been based on results from different methods of quantification. As a result, finding a commonality for the role of protein in foam quality has been a challenge.

Foam Analysis

Foam height and stability contribute to foam quality. Height is measured from the base of the foam, where it touches the wine, to its highest point; stability refers to the time it takes for the bubbles to collapse into the collar. There are many foam stability tests as no standard method has been universally accepted. (Andrés-Lacueva et al. 1996, Schramm 2005). Available analytical instruments include the Mosalux apparatus (Maujean et al. 1990), the Computerized Artificial Viewing Equipment (CAVE) and Fizzeye-Robot (Condé et al. 2017b) and the Krüss Dynamic Foam Analyzer DFA100 (Krüss USA, Matthews, NC).

Factors Affecting Foam Height and Stability

There are several factors that affect sparkling wine foam height and stability including grape cultivar, base wine composition (e.g., grape protein concentration, grape maturity, malic acid concentration and level of alcohol), winemaking techniques, the use of certain fining agents (e.g., bentonite), and grape diseases.

Base wine composition is of considerable importance to the foaming of sparkling wine (Pozo-Bayón et al. 2009), as the grape cultivar can affect foam formation due to variations in protein and malic acid concentration (Liu et al. 2006, Pozo-Bayón et al. 2009, Schramm 2005, Wen et al. 2014). Harvest timing is also of interest since total grape protein is correlated to the grape maturity (Liu et al. 2018). Carbonation method can have an effect; in one study, Méthode Traditionelle wines were found to contain, on average, 2-fold higher protein concentrations compared to sparkling wines made via other methods (Culbert et al. 2017). This was due to mannoproteins derived from the cell wall during alcoholic fermentation, and released into the

wine via autolysis during bottle aging (Esteruelas et al. 2015). Most studies show a positive correlation to protein concentration, whether yeast or grape-derived. (Brissonnet and Maujean 1993, Pozo-Bayón et al. 2009).

Although there seems to be a positive correlation between protein concentration and foam formation, the impact on foam stability has shown contradictory results (Martínez-Lapuente et al. 2015). Proteins that promote foam formation may not necessarily improve foam stability as foam-promoting proteins tend to be more flexible and have lower molecular weights than foam stabilizers (Condé et al. 2017a). Mannoproteins are good foam stabilizers, as they are hydrophobic and tend to have higher molecular weight, better stabilizing the CO_2 bubbles in the foam (Blasco et al. 2011).

Grape proteins, while having a positive effect on foam characteristics, also present particular challenges. Proteins like TLPs can cause the appearance of a protein haze in the wine, a fault that could render a wine un-saleable (Esteruelas et al. 2015). To eliminate this risk, winemakers often treat white wine with bentonite, an additive primarily composed of clay minerals. Bentonite has a negative charge and will adsorb positively charged protein molecules and precipitate out of solution (Jaeckels et al. 2017). While there is evidence that chitosan can be effective at eliminating protein haze in wines after bottling (Colangelo et al. 2018), bentonite is more widely used (Van Sluyter et al. 2015).

Stabilization treatments during the different stages of sparkling wine production, such as bentonite addition, have been shown to remove around 75% of wine protein and peptides (Culbert et al. 2017). Gel filtration by FPLC showed that bentonite selectively removed the 60 kDa and 20–30 kDa protein fraction while the higher molecular fraction was not affected (Vanrell et al. 2007). This is important since TLPs have a molecular weight of ~24 kDa

compared to mannoproteins which range from 10 to 200 kDa (Blasco et al. 2011). Adding bentonite either to the base wine as a fining agent or to the bottle as a riddling agent can greatly affect the foaming ability of base wines by reducing the maximum height and persistence of the wine foam (Lira et al. 2014, Vanrell et al. 2007).

Malic acid concentration in the base wine is positively associated with foam height (Andrés-Lacueva et al. 1997). Typically, tartaric and malic acids account for 90% of the acids found in grapes, with tartaric acid predominating (Lamikanra et al. 1995). The ratio of malic acid to tartaric acid varies greatly with cultivar (Liu et al. 2006) and native grapes tend to have higher acidity than *V. vinifera* grapes (Wen et al. 2014). As the grape ripens, the malic acid naturally degrades. The rate of this degradation is dependent on temperature; as temperature increases, so does the rate of malic acid degradation (Lamikanra et al. 1995, Sweetman et al. 2014).

High malic acid concentration, caused by growing conditions or genetic parentage is the main contributor to high titratable acidity in hybrid grapes (Gallander 1977). Though positively associated with foam characteristics (Liu et al. 2006), malic acid can have an unfavorable influence on the sensory properties of wine, such as overpowering tastes of tartness and astringency (Gallander 1977). To rectify this, winemakers often de-acidify the wine either through acid neutralization via a calcium carbonate addition, or via biological malolactic fermentation, a process that converts malic acid into lactic acid. Lactic acid has been found to favor foam stability (López-Barajas and Lopez-Tamames 1998), rather than height. This suggests that these winemaking techniques to reduce malic acid must be done with care if maximum foam height is desired (Andrés-Lacueva et al. 1997).

Ethanol is the principal molecule responsible for the value of the CO_2 diffusion coefficient (Bonhommeau et al. 2014) and lower alcohol concentrations are reported to have a

positive effect of foam stability. This is due to the increased activity of the surfactants and their ability to be adsorbed at the gas-liquid interface (Kemp et al. 2018). There is a competition for adsorption between alcohol and other molecules at the gas-liquid interface; the lower the concentration of alcohol, the greater the ability of other molecules to be adsorbed (Dussaud et al. 1994). It must be noted, though, that the negative effects of higher alcohol on foam formation could be counteracted by the mannoproteins produced by yeast autolysis in the second fermentation in the bottle (Andrés-Lacueva et al. 1997).

Lastly, diseases that target grapes, like *Botrytis cinerea,* are of particular interest; several studies have shown that grapes infected with *B. cinerea* lead to a decrease of wine foaming properties (Andrés-Lacueva et al. 1997). *B. cinerea* is a wide-spread fungal pathogen, responsible for gray mold disease (Cilindre et al. 2007). The 'foam-active' protein fraction in the grape is altered by the presence of the fungus. The proteins could be partially or completely degraded by fungal proteases while other proteins seem to be synthesized either by the fungus or by the plant's defense mechanisms (Cilindre et al. 2007). One study showed that when compared to a sparkling wine made from healthy grapes, the foam height and foam stability of the sparkling wine made from botrytized grapes was reduced by 47.7% and 33.3%, respectively (Cilindre et al. 2007).

It must be acknowledged, though, that foam formation and stability is complex and is dependent on an interplay of a variety of compounds rather than any one compound in particular (Andrés-Lacueva et al. 1996).

Purpose of this study

Recent work on red hybrid grapes show that non-*vinifera* grapes species have significantly higher TLP concentrations than do red *V. vinifera* cultivars (Springer et al. 2016), but protein

concentration in hybrid and native white cultivars has not been investigated. The purpose of this study is to begin to characterize the concentration of TLPs and malic acid in sparkling base wines with their foaming properties. Though there are three criterion of foam properties – foam height, foam stability (duration) and collar – the scope of this study focuses on the height and stability of the foam formed on the surface of the wine.

Materials and Methods

Wines

Fifteen commercial sparkling wines produced from native or inter-specific hybrid grapes, including one produced from *Vitis vinifera*, were sourced from the Finger Lakes, New York, wine region. Seven were monovarietal sparkling wines and the remaining eight sparkling wines are blends of interspecific hybrids and/or native grapes. Information about processing method was provided by the producer of each wine (Table 1).

Table 1. Characteristics of sparkling wines sourced from the Finger Lakes region of New York.

Sample Code	Cultivar	Carbonation Method[1]					Fining Agent[2]	
		c	f	r	t	tr	a	b
F1	Cayuga white		*					
F2	Niagara		*					
F3	Niagara		*					
F4	80% Catawba, 20% Red hybrid blend		*					
F5	60% Vidal blanc, 25% Traminette, 15% Siegfried		*					
F6	90% Catawba, 10% Chambourcin		*					
F7	96.5% Catawba, 3.5 % Vincent		*					*
F8	Valvin Muscat		*					*
C1	78% Catawba, 10% Diamond, 10% Isabella, 2% Vincent	*						*
C2	85% Diamond, 15% Golden Muscat	*						*
T1	Catawba				*		*	
T2	50% Concord, 50% Cayuga white				*			
T3	Marquette				*			*
TR4	Chardonnay					*		*
R1	Edelweiss			*				

[1]Carbonation method; c (Charmat), f (forced); r (referment); t (traditional); tr (transfer method)
[2]Fining agent, a (Adjuvant83–a blend of bentonite and minerals), b (bentonite)

Reagents

For protein extraction, a 2M solution of potassium chloride (KCl) (VWR Amresco Lifescience Solon, OH) and a 10% solution (w/v) of sodium dodecyl sulfate (SDS) in deionized water (MP Biomedicals Solon, OH) were produced as described in Gazzola et al. (2015).

Analytical Methods

Foam Parameters

Foam height (HM), the maximum height reached by the foam column, and foam stability (TS), the time of 50% foam collapse (T_{50}), were analyzed with the Krüss Dynamic Foam Analyzer DFA100 (Krüss USA, Matthews, NC) using a modified form of a method described by Oetjen et al. (2014). Following 1hr of vacuum degassing, 26 mL sample aliquots were put into the 20mm ID glass analysis cylinder and nitrogen injected at a rate 0.5L min^{-1} through a glass frit (16–40 μm pore size) at the bottom.

Foam height with gas injection was measured with a line sensor (5 frames sec^{-1}) and recorded for 120 seconds. Once gas injection ceased, foam collapse was measured (2 frames sec^{-1}) and recorded for 60 seconds. Experiments were performed at ambient temperature, i.e., 20 ± 1 °C. All parameters were analyzed in triplicate.

Malic Acid Concentration

The malic acid concentration of each of the sparkling wine samples was determined with the use of the Oenofoss FTIR (FOSS North America, Eden Prairie, MN).

Protein Concentration

Protein was extracted using a modified KDS precipitation method (Fusi et al. 2010, Vincenzi et al. 2005). Ten μL of the SDS solution was added to 1 mL of wine sample and was heated at 100°C for 5 min in a heating block. Next, 250 μL of the 2M KCl solution was added and the mixture incubated for 45 minutes at 4°C. After incubation, the protein pellet was collected by centrifugation at 14,000g for 15 minutes at 4°C, then washed once with 1 mL of the 2M KCl solution.

Protein Quantification

Protein pellets were resuspended in 1mL of DI water and heated for 5 minutes to improve solubilization. The BCA-200 Protein Assay Kit (Thermoscientific, Rockford, IL), which is based on the method described by Smith et al. (1985), was used to ascertain the protein concentration. Sample absorbance was measured at 562 nm with a Spectronic Genesys 2 (Thermoelectron Corp., Madison WI). Calibration curves were obtained by using known concentrations of BSA dissolved in distilled water.

Statistical Analysis

Data analysis, modeling, and figure generation was performed using the statistical program JMP, version 14 (SAS Institute Inc., Cary, NC). Correlation, simple and multiple regression, ANOVA and t-tests were performed to guide further analysis. For effect of carbonation methods on foam, the one-way ANOVA analysis was used. Significant results were followed by post hoc comparisons using Dunnett's method with the traditional method of carbonation as the control. In addition, this same hypothesis was tested using non-parametric Kruskal-Wallis Tests (Supplemental Table 1). For all statistical analyses, the significance level of $P \leq 0.05$ was applied.

Results

Table 2. Mean values of foam variables and characteristics in 15 sparkling wines.

Wine sample[1]	Protein concentration (μg/ml)	Malic acid (g/L)	Maximum height (mm)	Height at sparging cease (mm)	Time to 50% collapse (s)[2]
f1	67.8	3.0	58.6	49.9	9.2
f2	243.0	1.2	146.2	90.9	8.5
f3	136.0	0.0	154.9	100.4	17.3
f4	62.9	1.5	82.3	57.0	16.3
f5	222.4	2.3	73.7	55.4	8.5
f6	91.1	1.9	173.9	104.7	10.0
f7	188.0	3.5	70.8	59.2	6.3
f8	288.0	5.0	114.2	99.43	14.2
c1	174.0	1.6	71.4	46.6	7.8
c2	179.0	1.5	127.4	77.0	8.7
t1	31.90	0.0	44.3	31.9	5.8
t2	64.1	0.0	41.4	23.9	4.2
t3	10.5	3.2	74.0	46.9	17.8
tr4	53.0	0.1	30.9	13.2	1.2
r1	109.0	0.0	82.6	84.2	10.0

[1]Carbonation method: c (Charmat); f (forced); r (referment); t (traditional); tr (transfer method)
[2]Estimated to closest data point

Foam parameters

Maximum Height

There was a significant interaction effect of protein and malic acid concentration on maximum height (F stat 5.679, p-value 0.022). At high protein concentrations, increases in malic acid concentration decreased the maximum height achieved by the foam. However, at high malic acid concentration, an increase in protein concentration had no effect on maximum height. The nature of this interaction is described by the interaction profile graph (Figure 1).

Height at cease sparging

There was a significant positive relationship (F stat 17.801, p-value 0.0001) between protein concentration and the height at cease sparging (Figure 2A). For every unit increase in protein concentration, the height at sparging cease is expected to increase by 0.190mm, on average (r = 0.541). However, the regression of height at sparging cease on malic acid was not significant (F stat 3.575, p-value 0.065, r = 0.277). Lastly, the multiple regression of height at sparging on protein concentration and malic acid concentration showed that protein concentration was significant (F stat 12.860, p-value 0.0009); malic acid was not significant (F stat 0.024, p-value 0.879).

Time to 50% Foam Collapse

The relationship of T_{50} on protein concentration was not significant (F stat 0.038, p-value 0.847, r = 0.030), though malic acid (Fig 2B) showed a relationship approaching significance (F stat 3.999, p-value 0.052). For every one unit increase in malic acid concentration, the T_{50} increased by 0.99 seconds, on average (r = 0.292). Later, the regression of collapse on protein concentration and malic acid concentration showed that the malic acid is significant (F stat 4.673, p-value 0.036) whereas the protein concentration was not a significant factor. The average time it takes for T_{50} is expected to increase by 1.223 seconds, on average, for every unit increase in malic acid concentration.

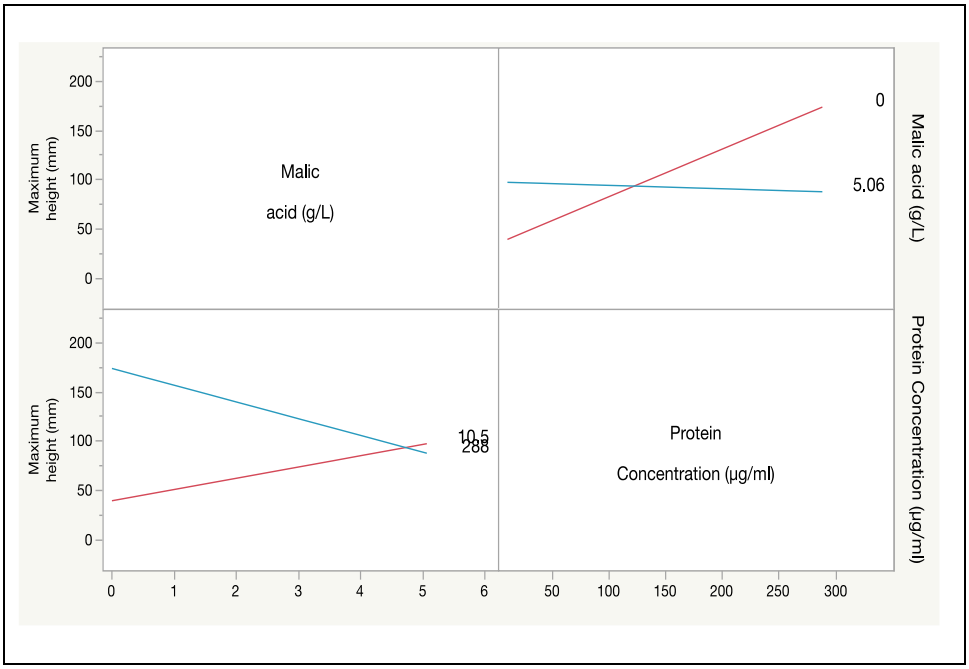


Figure 1. Interaction effect of protein and malic acid concentrations on foaming parameters of 15 wines as analyzed by Krüss Dynamic Foam Analyzer DFA100.

Effect of carbonation methods on foam characteristics

Maximum height

Carbonation method had a significant effect (F stat 6.398, p-value 0.0004) on foam HM (Fig. 3A). According to the post hoc comparison using Dunnett's Method, only the forced carbonation method height differential was significant. The foam of the forced carbonated wines measured, on average, 56.11 mm (p-value 0.0009) taller than the traditional method wines. The Charmat method foam measured 46.21 mm taller (p-value 0.063), refermentation was 29.4 mm higher (p-value 0.57) and transfer method 22.26 mm shorter (p-value 0.77) than the traditional method though these were not significant.

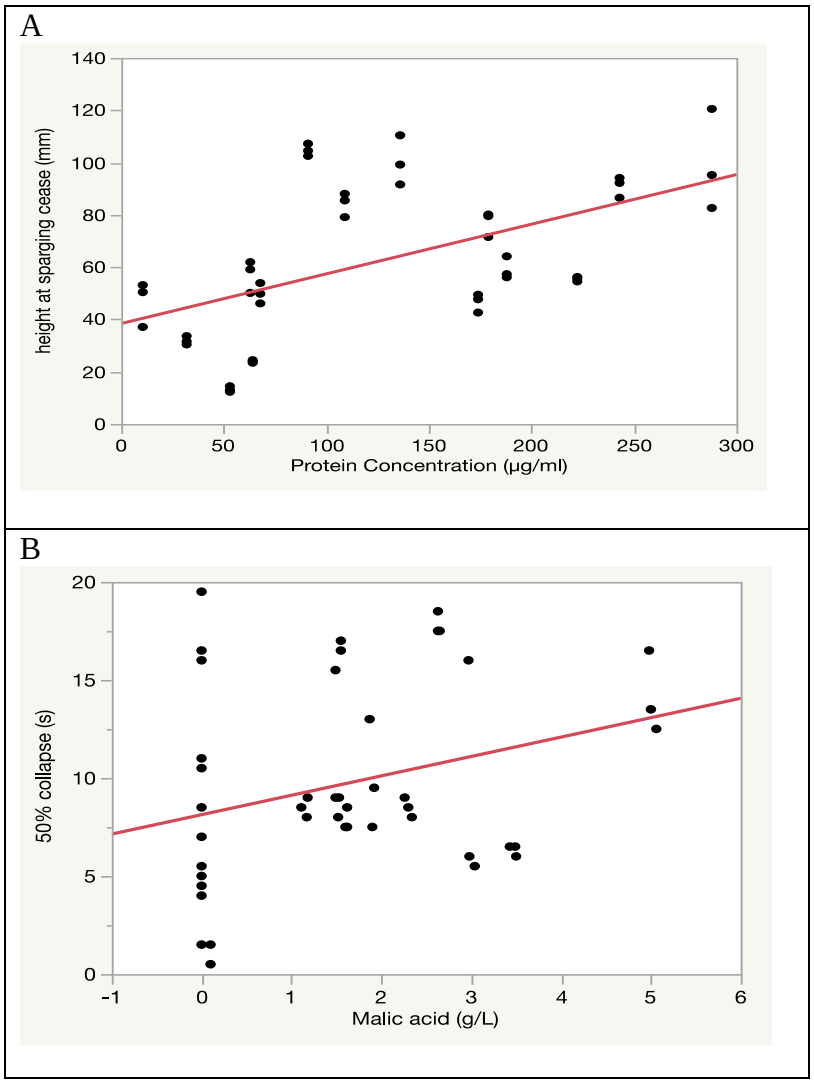


Figure 2. Correlations between foaming parameters of 15 wines as analyzed by Krüss Dynamic Foam Analyzer DFA100; A**.** Protein concentration on height at cease sparging. B. Malic acid concentration on T_{50}.

Height at sparging cease

The height of the foam at sparging cease was significantly affected by the carbonation method (F stat 13.679, p-value <0.0001) (Figure 3B) as post hoc comparisons with the traditional method showed that the foam in the refermented wine was 49.989mm taller (p-value 0.0017), forced carbonation was 42.918mm taller (p-value <0.0001) and Charmat was 27.589mm taller (p-value 0.391) than the traditional carbonation method's foam height at sparging cease. The transfer method height was not significantly different.

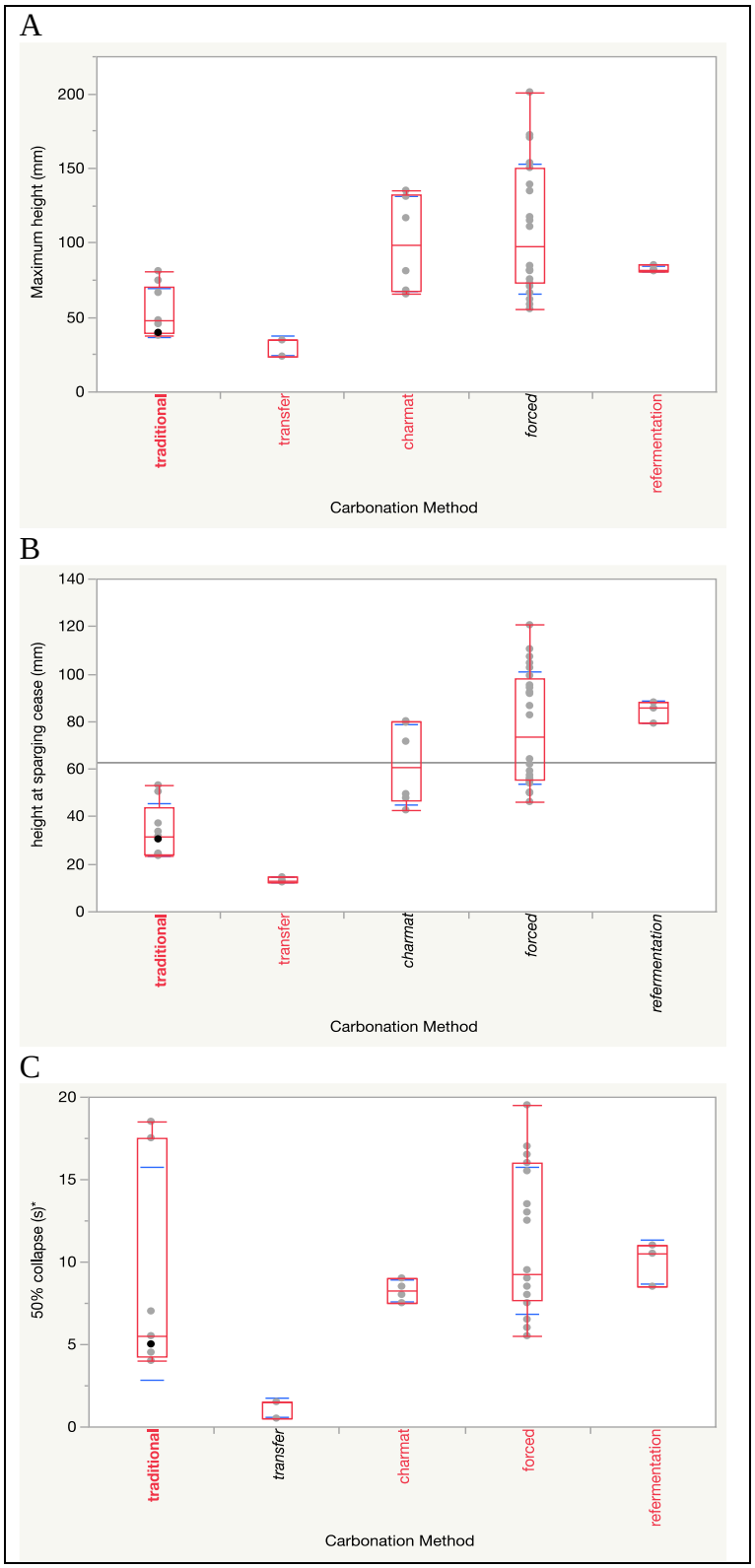


Figure 3. Comparison of carbonation methods to traditional method; A. Carbonation effects on maximum height; B. Carbonation effects on height at sparging cease. C. Carbonation effects on time of 50% foam collapse.

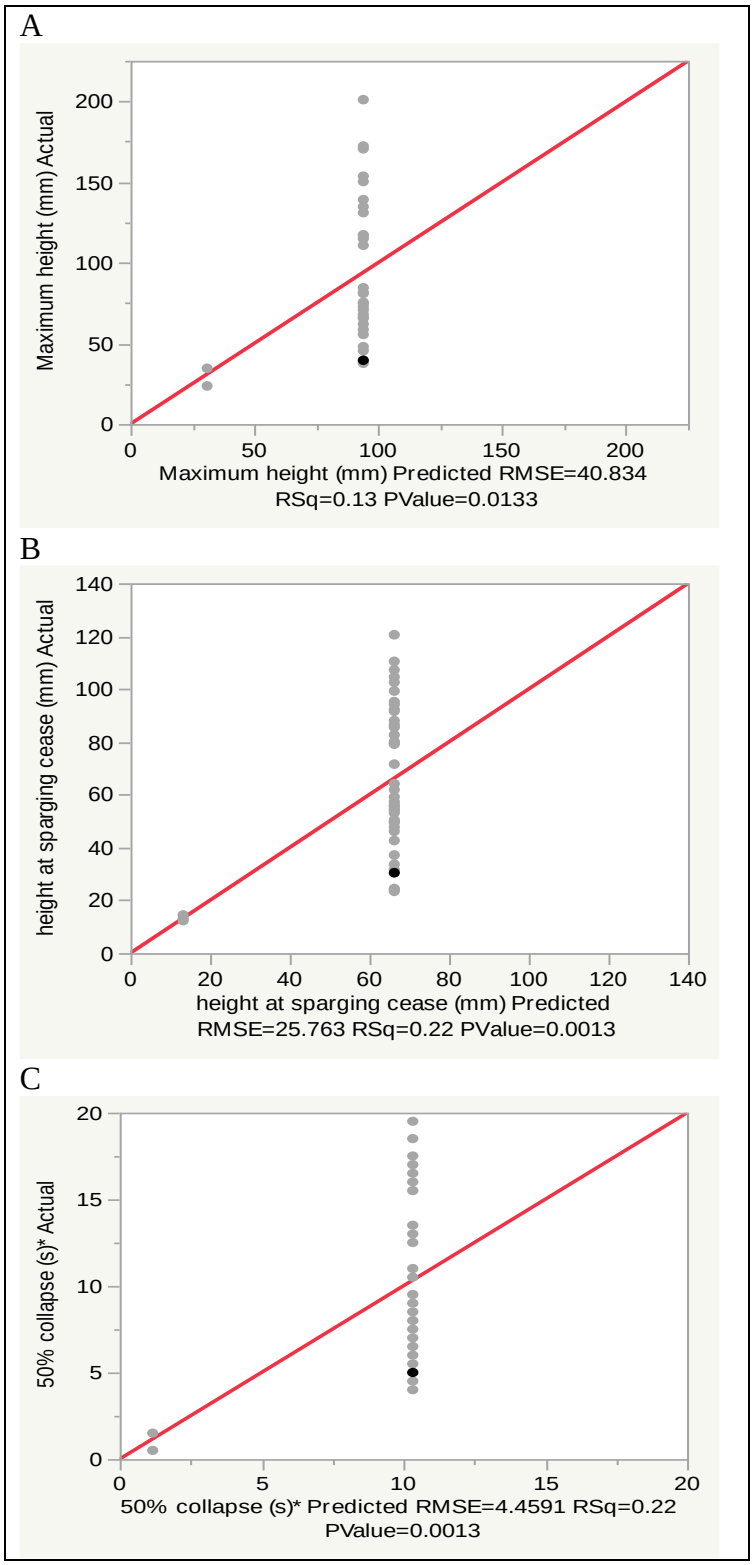


Figure 4. Comparison of foaming parameters between *V. vinifera* and non-*V. vinifera* sparkling wines; A. Difference in Maximum height achieved. B. Difference in height at sparging cease. C. Difference in rate of 50% collapse.

Time to 50% foam collapse

Though the carbonation method had a significant effect (F stat 3.68 p-value 0.012) on T_{50} (Figure 3C), the post hoc comparison to the traditional method showed that only the transfer method at 8.11 seconds faster was significant (p-value 0.0340). The forced carbonation T_{50} took 2.01 seconds longer (p-value 0.6338), the refermentation took 0.72 seconds longer (p-value 0.9979) and the Charmat method T_{50} took 1.03 fewer seconds (p-value 0.9811) than the traditional method.

Comparison of V. vinifera and non-V. vinifera on Foam Characteristics

Protein concentration

Protein concentration was not significantly different between *V. vinifera* and non-*V. vinifera* sparkling wines. (F stat 2.807, p-value 0.101)

Malic acid concentration

Between the *V. vinifera* and non-*V. vinifera* sparkling wines, the malic acid concentrations were approaching significance (F stat 3.80, p-value 0.0579), with a difference of 1.64 g/L.

Maximum height

The maximum height achieved by the foam was significantly different (F stat 6.674 , p-value 0.013) between the *V. vinifera* and non-*V. vinifera* sparkling wines (Figure 4A), with a difference of 63.046 mm.

Height at Sparging cease

Between *V. vinifera* and non-*V. vinifera* sparkling wines, the height of the foam at sparging cease was significant (F stat, p-value 0.0013) with the difference of 53mm (Figure 4B).

Time to 50% foam collapse

The T_{50} difference of 9.16 seconds between *V. vinifera* and non-*V. vinifera* sparkling wines was significant (F stat 11.83 , p-value 0.0013) (Figure 4C).

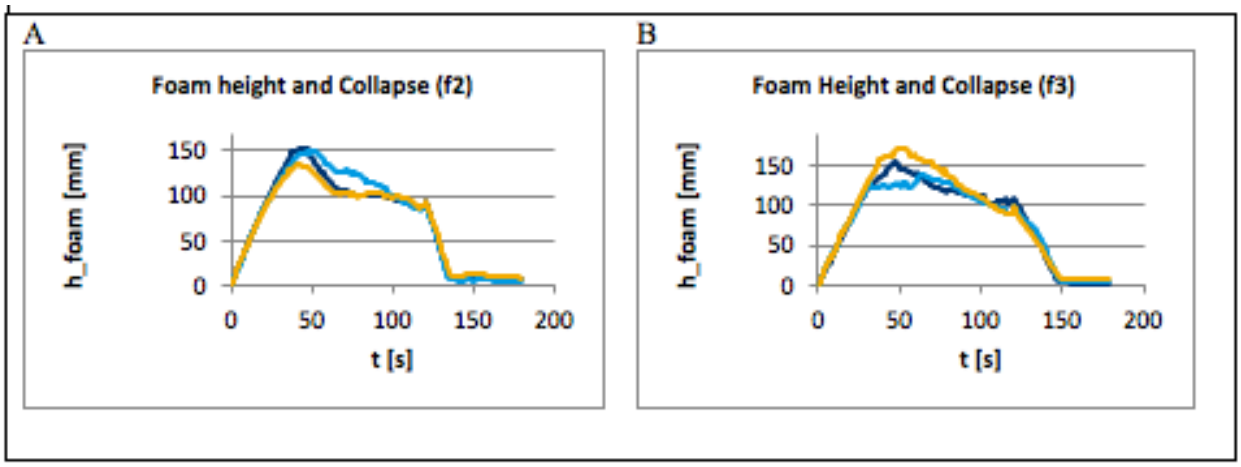


Figure 5. Foam analysis data of sparkling wines made from Niagara grapes as analyzed by Krüss Dynamic Foam Analyzer DFA100.

Discussion

Effects of protein and malic acid on foam height

To date, literature discussing the influence of protein concentration in sparkling base wines has established a positive correlation between protein and HM (Condé et al. 2017a) and between malic acid concentration and HM (Andrés-Lacueva et al. 1997). In contrast, the current study suggests that the interaction of protein and malic acid concentration, rather than individual effects of either, had the strongest influence on HM (Figure 2).

At high protein concentration, increases in malic acid decreased HM while at high malic acid concentration, increased protein content had no effect. This suggests that the malic acid acts to diminish the contribution of the protein to the HM. These findings contradict those of Andrés-Lacueva et al. (1997) who found that both protein and malic acid were positively correlated with HM, but are supported by Liu et al. (2018) who reported that more mature grapes, which have

greater protein concentration and lower malic acid concentration, produce greater HM. This suggests that de-acidification techniques like malolactic fermentation may increase HM.

It is important to note that the protein and acidity of sparkling wine composition is directly related to grape cultivar and grape composition at harvest. Protein concentration in grapes is dependent on grape cultivar and grape maturity (Liu et al. 2018, Pozo-Bayón et al. 2009, Schramm 2005). Previous studies on red hybrid grapes show that non-*V. vinifera* grapes species have significantly higher TLP concentrations than do red *V. vinifera* cultivars (Springer et al. 2016). Recent work has shown that protein concentrations of white non-*V. vinifera* grapes range from 328 mg/L-1672 mg/L (Andrievsky and Mansfield 2019), however, it must be noted that no significant difference in protein concentration was observed here between *V. vinifera* and non-*V. vinifera* sparkling wines.

In this study, two of the forced carbonated native/hybrid blend sparkling wines were fined with bentonite (Table 1) and still maintained a higher protein concentration compared to bentonite-fined native and/or hybrid sparkling wines that were carbonated via secondary fermentation; this is notable, as the secondary fermentation would introduce yeast-derived mannoproteins, likely increasing protein concentration (Table 2). Since bentonite selectively fines proteins of 60 kDa and 20–30 kDa including grape-derived TLPs and excluding most mannoproteins (Blasco et al. 2011, Vanrell et al. 2007), this suggests that some grape cultivars naturally have higher protein concentrations, or that cultivars used in forced carbonated wines were harvested at greater grape maturity, resulting in higher protein concentrations.

The effect of malic acid was difficult to characterize in this work due to other wine chemistry variables. Some of the wines lacked malic acid, presumably due to malolactic fermentation. For example, though f2 and f3 are both composed of 100% Niagara grapes, f2 has 1.2 g/L malic acid,

and f3, none (Tables 1&2). As expected from lower malic acid and 44% more protein (Table 2), f2's HM is shorter than f3, though the height at sparging cease and the rate of 50% foam collapse are not significantly different, suggesting that other factors may play a role. In fact, these two sparkling wines have comparable foaming profiles (Fig. 5 A&B), suggesting that grape cultivar is indeed a contributor to foaming characteristics, though grape and wine chemistry must also be considered.

Previous studies report that protein content is also affected by the carbonation method of the sparkling wine. Those studies show that sparkling wines made by traditional, Charmat and transfer methods had greater protein concentrations, in some cases up to 2-fold higher than the forced carbonation method (Culbert et al. 2017), due to yeast-derived mannoproteins introduced to the wine during second fermentation. This suggests a synergistic interaction between yeast mannoproteins and grape-derived proteins.

In contrast, the forced carbonated wines in this study achieved statistically higher HM than traditional method wines (Fig. 3A) but the importance of this finding is complicated by the fact that protein content of the base wines of the traditional, Charmat and transfer methods were not consistent (Table 1). Four of the five wines carbonated via a second fermentation were fined with bentonite or another similar fining agent, which previous studies have found to remove up to 75% of protein in a sparkling wine (Culbert et al. 2017). Adding bentonite as a fining or riddling agent may significantly affect wine characteristics by reducing the maximum height and persistence of the sparkling wine foam (Lira et al. 2014, Vanrell et al. 2007).

Protein and malic acid on foam stability/ duration of foam collapse

In this work, wine foam at cease sparging and T_{50} were impacted by only protein or malic acid, respectively (Fig. 2). These data agree with previous studies showing that protein

promoting foam formation doesn't necessarily improve foam stability (Condé et al. 2017a) and that higher malic acid concentration had positive correlation with foam height and stability (Andrés-Lacueva et al. 1996).

Foam duration is directly related to bubble stability, and stability is itself dependent on the composition of the film that supports it. Yeast-derived mannoproteins have been identified as foam stabilizers since they are hydrophobic and tend to have higher molecular weight, which stabilizes CO_2 bubbles in the foam (Blasco et al. 2011). Here, only foam on the transfer method sparkling wine collapsed significantly faster than the traditional method (Fig. 3C), but since it was also only wine made with *V. vinifera*, it is difficult to base any conclusions on this observation.

Lastly, it should be noted that the wines in this preliminary survey are from different producers and have different chemical compositions. This work provides an initial exploration of the interactions between protein content and malic acid levels on foam parameters however, these interactions are complex and affected by wine composition, which is in turn affected by winemaking techniques. These interactions need to be tested in sparkling wines of the same chemical composition elaborated under different production methods to fully understand the effect of sparkling wine composition on sparkling wine foam characteristics.

Conclusion

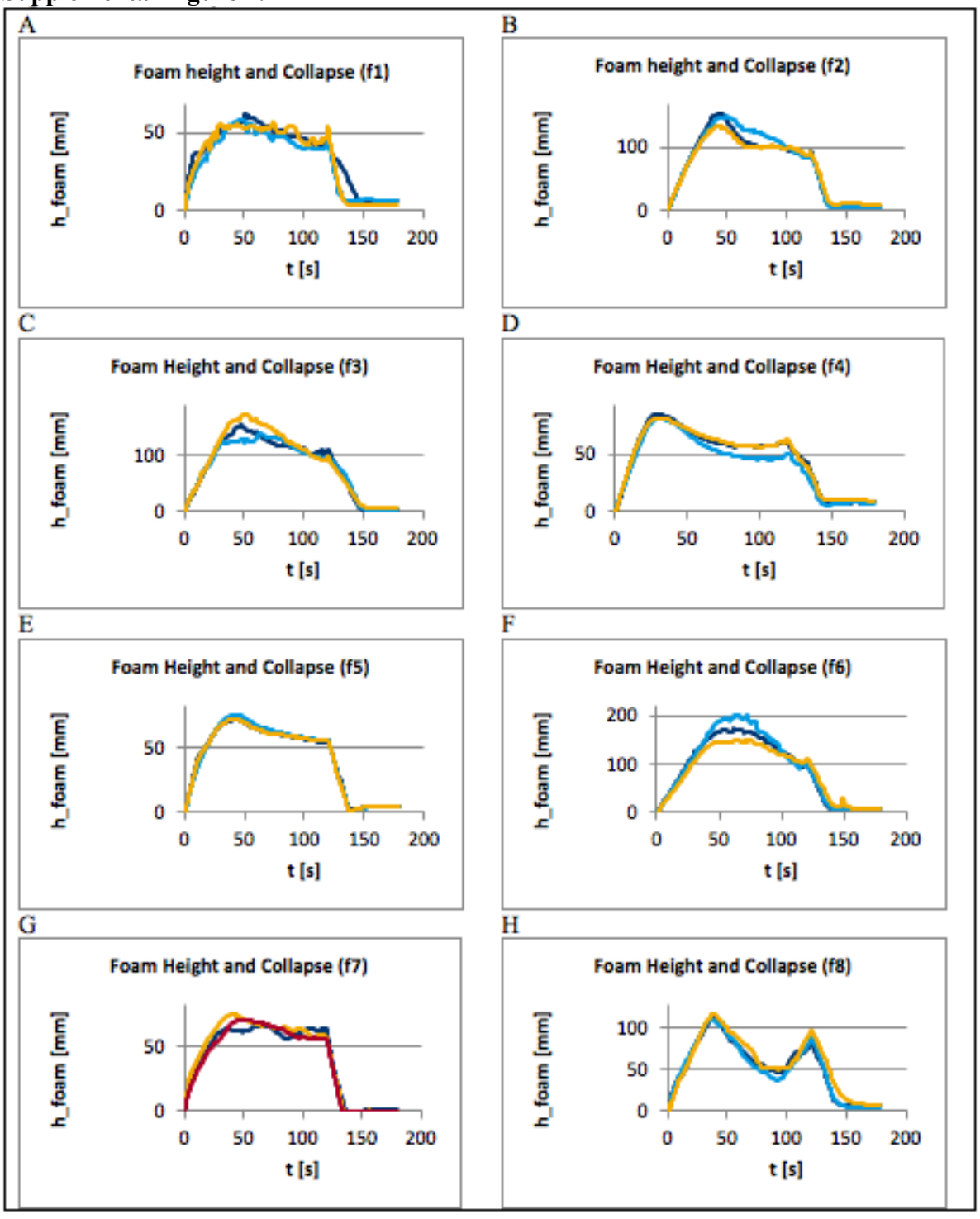
In this work, the interactive effect of protein and malic acid concentrations was found to have the greatest influence on foam height of sparkling wines made from non-*V. vinifera* grapes. Though previous studies have shown positive correlations between protein and malic acid concentrations on foam height and stability, this study found malic acid to have influence on foam stability that only approached significance. Sparkling wine production method showed the

greatest influence on foam characteristics, with forced carbonated sparkling wines achieving significantly taller foam height than those produced by the traditional method.

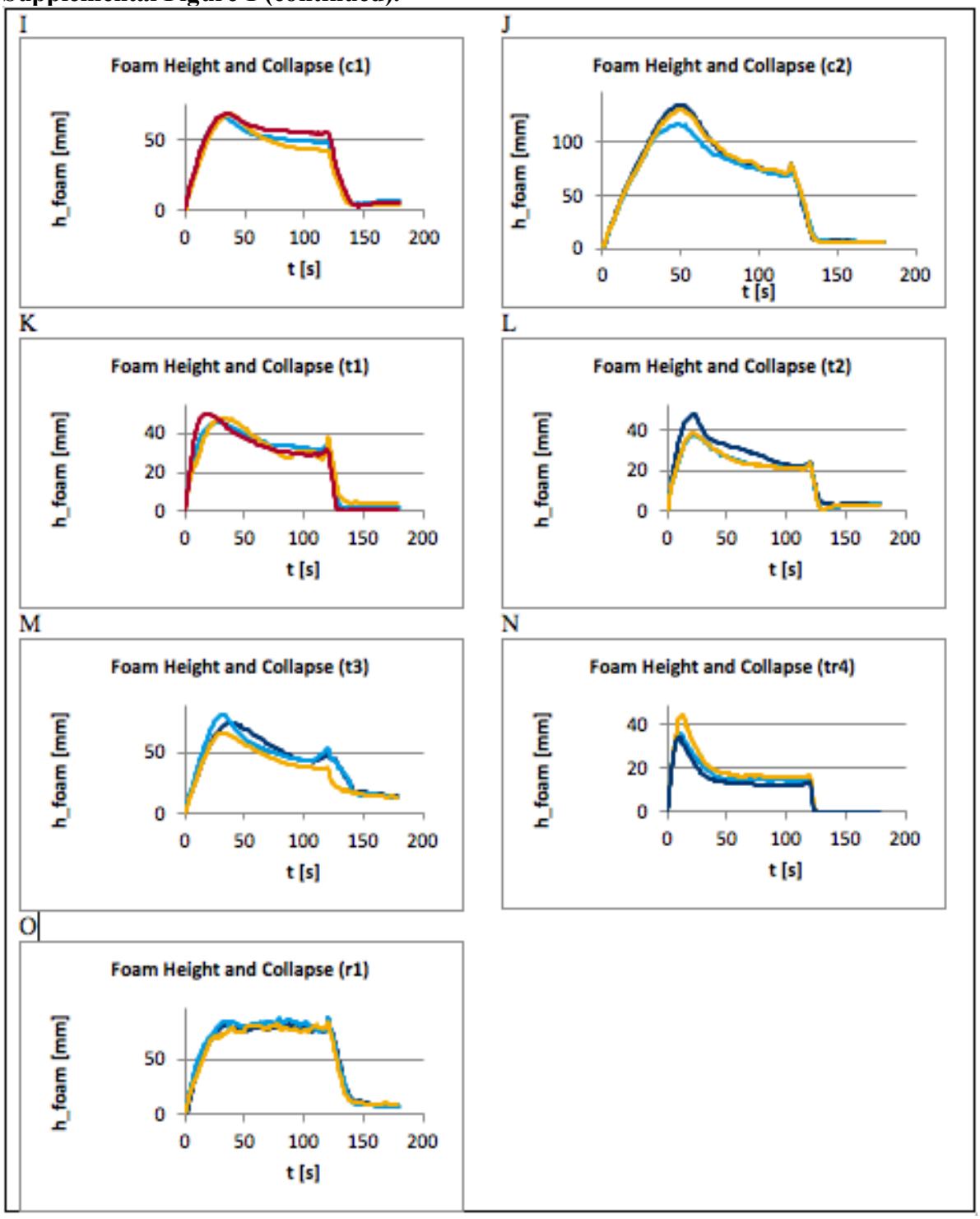
The sparkling wines studied were made from native and/or interspecific hybrid grapes from the New York Finger Lakes, and therefore, may be affected by variables like cultivar, harvest maturity and carbonation method. To fully understand the importance of each parameter, studies building on these initial findings are called for. Characterizing and understanding these impacts, however, should prompt regional winemakers to start considering production choices for sparkling wines produces from non-*vinifera* cultivars.

Appendix

Supplemental Figure 1.



Supplemental Figure 1 (continued).



Supplemental Figure 1. Foam analysis data of 15 sparkling wines as analyzed by Krüss Dynamic Foam Analyzer DFA100; A. 100% Cayuga white; B. 100% Niagara; C. 100% Niagara; D. 80% Catawba, 20% Red hybrid blend; E. 60% Vidal blanc, 25% Traminette, 15% Siegfried; F. 90% Catawba, 10% Chambourcin; G. 96.5% Catawba, 3.5 % Vincent; H. 100% Valvin muscat; I. 78% Catawba, 10% Diamond, 10% Isabella, 2% Vincent; J. 85% Diamond, 15% Golden muscat; K. 100% Catawba; L. 50% Concord, 50% Cayuga white; M. 100% Marquette; N. 100% Chardonnay; O. 100% Edelweiss

Supplemental Table 1. Non-parametric Kruskal-Wallis test comparing parameters of wines made with other carbonation methods to traditional method.

Parameter	Chi Square	DF	Prob>ChiSq
Protein Concentration	24.3310	4	<0.0001
Malic acid concentration	13.8515	4	0.0078
Maximum height	20.7092	4	0.0004
Height at sparge cease	25.9452	4	<0.0001
Time to 50% collapse	11.0666	4	0.0258

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