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Research article

Using synchronous fluorescence to investigate compounds and interactions influencing foam characteristics in sparkling wines

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Abstract: The appearance of bubbles and foam can influence the likeability of a wine even before its consumption. Since foams are essential to visual and taste attributes of sparkling wines, it is of great importance to understand which compounds affect bubbles and foam characteristics. The aim of this work was to investigate the effect of interactions among proteins, amino acids, and phenols on the characteristics of foam in sparkling wines by using synchronous fluorescence spectroscopy techniques. Results has shown that several compounds present in sparkling wines influence foam quality differently, and importantly, highlighted how the interaction of those compounds might result in different effects on foam parameters. Amongst the results, mannoproteins were found to be most likely to promote foam and collar stability, while phenols were likely to increase the ratio of small bubbles and collar height in the foam matrix. In summary, this work contributes to a better understanding of the effect of wine compounds on foam quality as well as the effect of the interactions between those compounds.

Keywords: effervescence, bubbles, protein, yeast invertase, foamability, wine quality.

1. Introduction

The visual appearance of a wine is the attribute that provides the first impression for the drinker. In the case of sparkling wines, since the vast majority result from secondary fermentation of white wines, bubbles and foams are of even greater importance. The appearance of bubbles and foam are judged subjectively by wine appreciators, which might set the likeability of the wine even before its consumption. Furthermore, bubbles' size and distribution influence foam texture, thus influencing the sensory experience of the wine consumer. Therefore, since foams alter visual, aroma and taste attributes of sparkling wines, it is essential to understand which compounds affect bubble and foam quality. Understanding the complex mechanism of foam and bubble formation, stabilization, and how it relates to overall wine quality will provide tools for wine producers and researchers to optimize wines with desired and improved qualities.

Proteins are commonly accepted as the leading wine compounds influencing foam quality [1,2]. To be able to form a foam, proteins need to be rapidly adsorbed and unfolded at the gas/liquid interface, whilst to promote stability, it is necessary to create a robust and flexible film able to reduce gas permeability and bubble coalescence [3]. Proteins that are flexible, able to expose more hydrophobic residues and reduce the average molecular mass are good candidates to promote foam formation [3]. On the other hand, proteins that resist mechanical deformation and can form intermolecular cross-linking are good candidates to promote stability [3]. During the second fermentation of sparkling wines produced by using the methode traditionnelle, amino acids, polysaccharides, peptides and proteins are released into the wine matrix [4]. Glycoproteins, such as mannoproteins and yeast invertase are proteins released by yeast cells during the second fermentation [4]. Mannoproteins found in wines vary in molecular mass from 53-560 kDa, and have a low ratio of protein/carbohydrate content, with mannose being the main carbohydrate component [5]. Vacuolar invertase originating from grapes, and yeast invertase resulting from wine fermentation are hydrophobic [2] and the most abundant proteins found in wines [2]. Since the majority of proteins found in sparkling wines are hydrophobic, it could be expected that most proteins found in wines are more likely to be involved in foam formation rather than foam stability. However, in solution, proteins might interact with other compounds through electrostatic and hydrophobic forces, hydrogen bonds and covalent linkages, and might bind to one another resulting in no free molecules [3]. Consequently, the protein's properties are modified, and so, the resulting effect on foamability and stability is unclear.

Phenolic compounds have an important role in wine quality, being involved in browning and bitter taste. They include all compounds with hydroxyl groups attached to aromatic rings [6]. Polyphenols have multiple phenol rings within a single structure, such as epicatechin [6]. They are subdivided as flavonoids and non-flavonoids. Flavonoids have a particular C6-C3-C6 3-ring structure with a central oxygen-containing ring [6]. Non-flavonoids are known as hydroxycinnamates and are the major class of phenols found in wines [6]. The influence of phenols in foam formation and stabilization of sparkling wines is largely unknown. It has been suggested that phenols might influence positively the foam of sparkling wines produced using red grapes, such as pinot noir [7]. On the other hand, although the interactions between phenolic compounds and proteins make it possible to eliminate the proteins responsible for haze formation [8], this could be detrimental to foam quality if it impairs the probability of the protein being able to act as a foam formation agent or stabilizer. The extent of proteins-polyphenols interactions is dependent on molecular size, number, and disposition of phenolic nuclei, conformational flexibility and water solubility of a specific polyphenol [9].

The formation and stabilization of bubbles and foam in sparkling wine is very complex and more likely to be the result of interactions among the several compounds present in the wine [10]. Nevertheless, the literature lacks knowledge regarding interactions between wine compounds and the effect these have on foam quality in sparkling wines. Fluorescence spectroscopy is a rapid method that can support the identification of compounds [11,12] and assessment of interactions in wine [13-15] and, more specifically, interactions between other compounds and proteins [16,17]. The aromatic amino acids tryptophan, tyrosine, phenylalanine are fluorophores found naturally in most proteins, with tryptophan being one of the most prevalent [18]. The fluorescence of tryptophan is highly dependent on the environment; thus, the study of the fluorescence pattern of tryptophan can assist in our understanding of changes in protein conformation and the interactions with other compounds [18].

Additionally, a survey study of the foaming parameters associated to sparkling wines elaborated by different production methods [19] raised several theories. Hence, several hypotheses were formulated: i) proteins and amino acids influence foam properties, such as foam stability; ii) compounds interact with proteins resulting in a positive or negative effect on foam quality; and iii) proteins (principally those originating from yeast) and amino acids increase the average foam lifetime, L_f.

To test these hypotheses, several compounds were added to a sparkling wine in order to isolate the effect on foam quality: i) yeast invertase, bovine serum albumin, asparagine, tryptophan; ii) yeast invertase + gallic acid, yeast invertase + asparagine; iii) yeast invertase. Then, to confirm whether compounds present in sparkling wines interact with proteins, fluorescence spectroscopic analysis was used. Finally, analysis of several foam parameters was performed, and the results obtained were assessed by statistical analysis.

2. Materials and Methods

Wine material. Sparkling wine samples were supplied by Domain Chandon, Yarra Valley, Victoria, Australia. The samples were composed of a sparkling white wine produced by following the méthode traditionelle, where a second fermentation is realized in the bottle and subsequently, the wine is subjected to a lees aging process [1]. The lees is a general name given to dead yeast cells that settle on the bottom of the vessel where the wine has been aging [4]. Wine aging duration is regulated by specific laws in countries such as France and Spain. Although, in Australia, there is no detailed laws regulating wine bottle aging as in Europe, the wines are aged for at least 9 months on lees to be classified as méthod traditionelle. The samples here studied had been aged for three months on lees prior to being analyzed, for research purposes only, and were not meant to be commercialized or consumed.

Wine samples. Chemical compounds (9) were added to the wine samples during the disgorging process, at a concentration of 100mg/L. The samples and abbreviations are summarized in Table 1.

Table 1. Summary of samples, chemical additions and corresponding abbreviations.

Sample	Concentration	Abbreviation
Control	n.a.	Ctrl
Alcohol	0.1% ABV	Alc
Yeast Invertase	100mg/L	INV
Bovine Serum Albumin	100mg/L	BSA
Manolees	100mg/L	MAN
Gallic Acid	100mg/L	Gall
Asparagine	100mg/L	Asn
Tryptophan	100mg/L	Trp
Yeast Invertase + Asparagine	100mg/L + 100mg/L	INVAsn
Yeast Invertase + Gallic Acid	100mg/L + 100mg/L	INVGall

Chemical additions. Albumin from bovine serum (≥ 96%) (Sigma-Aldrich Pty. Ltd., MO, USA), Yeast invertase from baker's yeast (≥ 300 units/mg solid[20]) (S. cerevisiae, Sigma-Aldrich Pty. Ltd., MO, USA); Mannolees (Lallemand); DL-asparagine (≥ 98%) (Thermo Fisher Scientific, UK); L-tryptophan (≥ 98%) (Sigma-Aldrich Pty. Ltd., MO, USA), gallic acid monohydrate (≥ 98%) (Sigma-Aldrich Pty. Ltd., MO, USA),

Determination of Foam Parameters. Foam parameters were obtained by analyzing 2 bottles of each treatment. Each bottle was analyzed, using a robotic pourer, according to the methodology previously described by Condé and colleagues [21]. The wines were at room temperature (18ºC) before pouring. The foam parameters quantified included: foam volume (V_f); foam time (F_t); average foam lifetime (L_f); collar height (h); foam velocity (F_v); drainability (D_r); percentage of wine in the foam (W_f); collar initial height (h_c); foam expansion (E); and small bubbles (S_b). The foam parameters quantified were the average of triplicate measurements per bottle.

Fluorescence Spectroscopy. Fluorescence spectroscopy coupled with parallel factor analysis is a cost-effective technique applied to assist identification of compounds in several food products, included wines [20,22]. Hence, the method was applied to facilitate the identification of possible compounds that could be further related to foam quality. Additionally, to investigate protein interactions, fluorescence emission measurements recorded with an excitation wavelength (λ_{ex}) of 278 nm were further examined. Tryptophan is excited around 280 nm and emits around 350 nm in proteins [23]. Furthermore, the fluorescence intensity (FI) was calculated by integration of the area under the curve in emission range λ_{em} 300-400 nm, following by λ_{ex} at 278 nm, for each sample.

The fluorescence excitation/emission matrices spectral data were obtained from each sample using a Varian Cary Eclipse fluorescence spectrophotometer operated in a synchronous scan mode using the front faced geometry (which has been shown to minimize issues associated with reabsorbance, inner filtering and scattering [24,25]) with the sample in a triangular quartz cuvette (path length 10 mm) mounted on a rotational mount to provide a 55° angle of the front face relative to the direction of the incident excitation beam. The use of the synchronous scanning mode reduces scattering originated from regions where λ_{em} ~ λ_{ex} [26]. The excitation range was set to 205 nm to 405 nm, and the corresponding synchronized emission was set to start at delta 10 nm (215 nm), with delta increments of 5 nm and the delta stop set to 200 nm (650 nm). Voltage was 800 volts to ensure low intensity fluorophores were recorded. All spectra were recorded at room temperature.

Statistical Analysis. Statistical data analyses, included the general linear model (Glm) and analysis of means (ANOM) were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and Minitab® 17.1.0 (Minitab Inc., PSU, PA, USA), respectively. Glm was used to assess whether there were any differences between each treatment and the control, for each parameter. ANOM provides visualization of the comparison for each group means against the overall mean, for each parameter.

Parallel factor analysis (Parafac). Each two-dimensional excitation/emission matrix (EEM) obtained from the fluorescence from each sample was overlaid into a three-dimensional array of data (X) with dimensions 'sample x emission x excitation'. The X matrix was further smoothed and analyzed using the Matlab Toolbox drEEM (Murphy et al. 2013). The selection of the number of components was based on the core consistency and percentage of explanation of the data. Subsequently, the excitation/emission ($\lambda_{ex}/\lambda_{em}$) patterns identified by Parafac analysis were further explored by principal component analysis in order to uncover chemical compounds that could possibly influence foam quality. Figures were obtained by using Minitab® 17.1.0 (Minitab Inc., PSU, PA, USA) and Matlab 2017b.

3. Results

3.1. Effect of the chemical additions on foam parameters.

Several foam parameters were analyzed, as previously described. The parameters F_t, L_f, h_c, W_f, E, did not show significant differences when compared to the control. The parameter V_f was significantly decreased by INV, BSA, Trp, INVAsn, and INVGall. The parameter F_v and D_r were also significantly decreased by Trp. Additionally, the parameter h was significantly increased by MAN, and S_b was significantly decreased by Asn. A summary of the parameters significantly different from the control sample, together with the direction of the effect on the parameter, is shown in Table 2.

Table 2. Foam parameters analyzed showing significant increase (↑) or decrease (↓).

Sample	V_f	F_v	D_r	h	S_b
Ctrl	na	na	na	na	na
Alc	n.s.	n.s.	n.s	n.s	n.s
INV	↓	n.s	n.s	n.s	n.s
BSA	↓	n.s	n.s	n.s	n.s
MAN	n.s	n.s	n.s	↑	n.s
Gall	n.s	n.s	n.s	n.s	n.s
Asn	n.s	n.s	n.s	n.s	↓
Trp	↓	↓	↓	n.s	n.s
INVAsn	↓	n.s	n.s	n.s	n.s

Furthermore, the average mean per treatment (sample), for each foam parameter was analyzed and compared between the group means, and significant differences (=0.05) were found for the parameters V_f, F_v, L_f, h, D_r, E, and S_b (Fig. 1). Figure 1 shows the different compounds assessed were found to influence differently those foam parameters. BSA was found to significantly increase L_f (Fig. 1). Asn and Gall significantly increased V_f, while Inv, InvGall and Trp decreased V_f, Asn was found to increase F_v and decrease S_b; MAN was found to increase h and E; Gall increased D_r and S_b (Fig. 1).

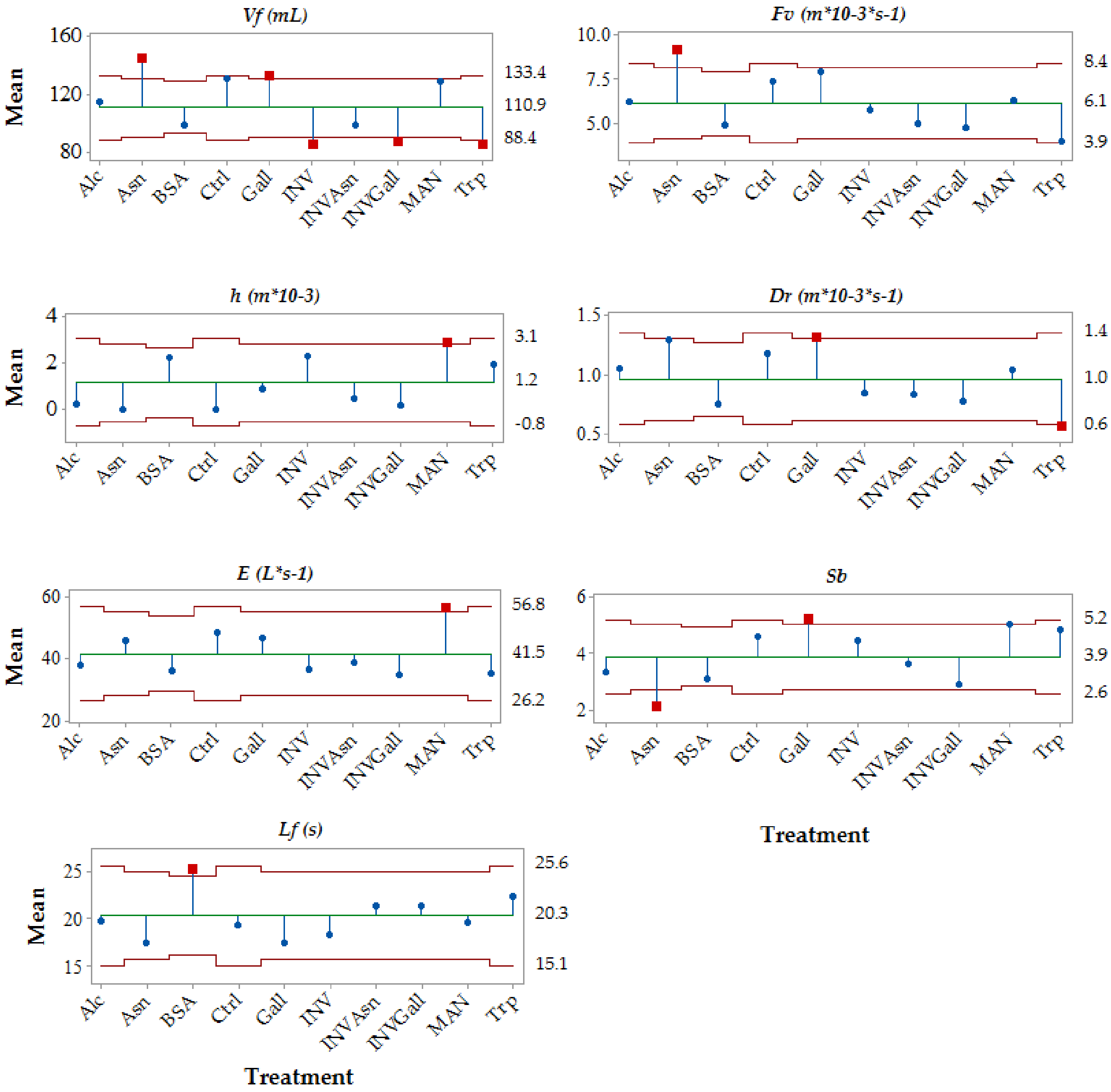


Figure 1. Group means of foam parameters. The average mean within a parameter, considering all samples, is represented by the green horizontal line; the means per sample is represented by the blue dot and when significantly different from the group means (α=0.05), the sample means is highlighted as a small red square situated outside the limits.

3.2. Spectrofluorescence and parafac analysis.

Contour plots were generated to assist visualization of the emission/excitation wavelengths and intensities for each sample, as well as identify possible interactions among the compounds. Figure 2 suggests a slight decrease in fluorescence emission for the samples Gall and INVGall, when compared to Ctrl, and an increase of fluorescence emission for the remaining samples. Hence, the preliminary analysis of the raw data suggested possible molecular interactions between the components present in the wine matrix might be responsible for the changes in fluorescence intensity.

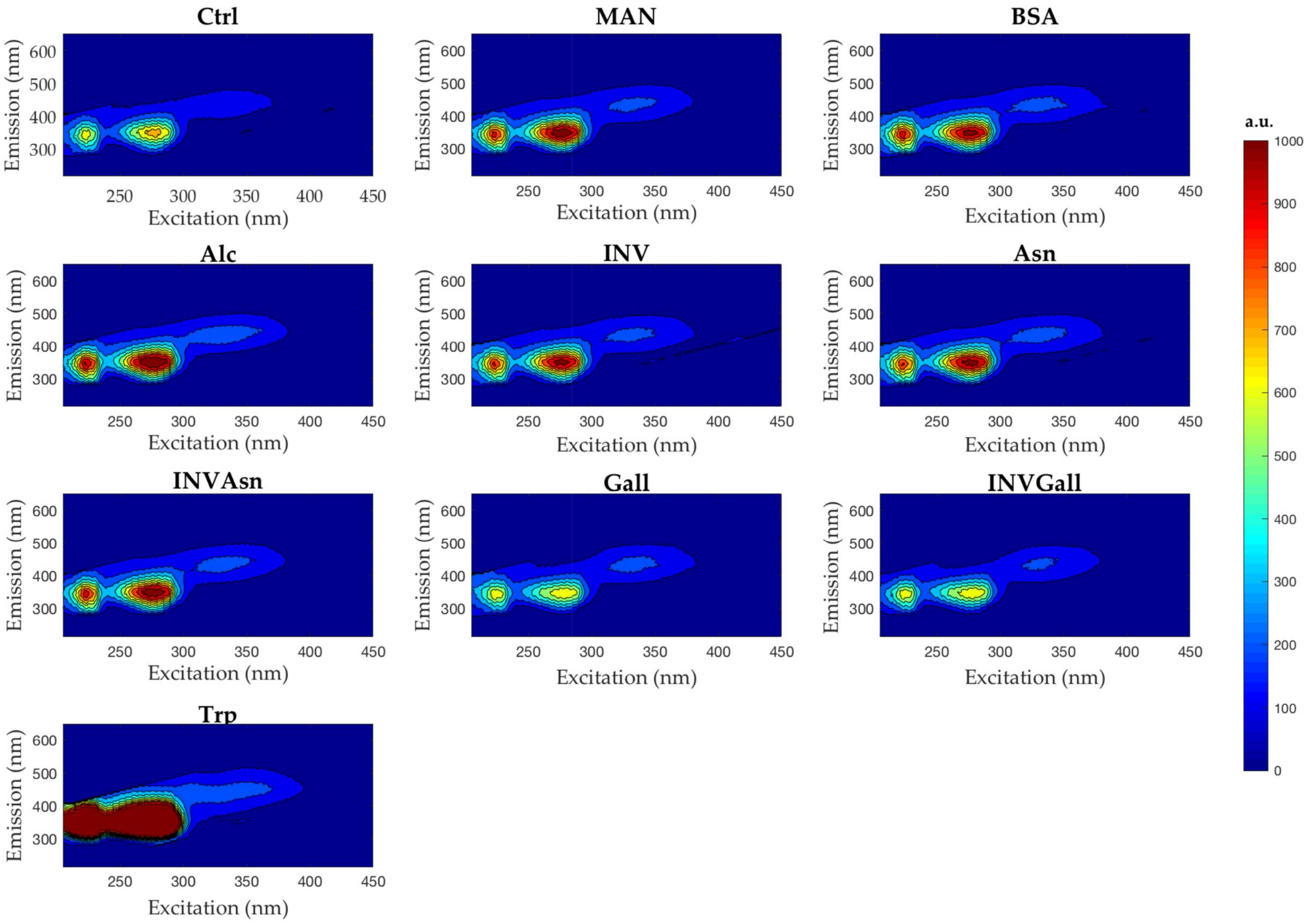


Figure 2. Excitation/emission contour plots of the samples analyzed.

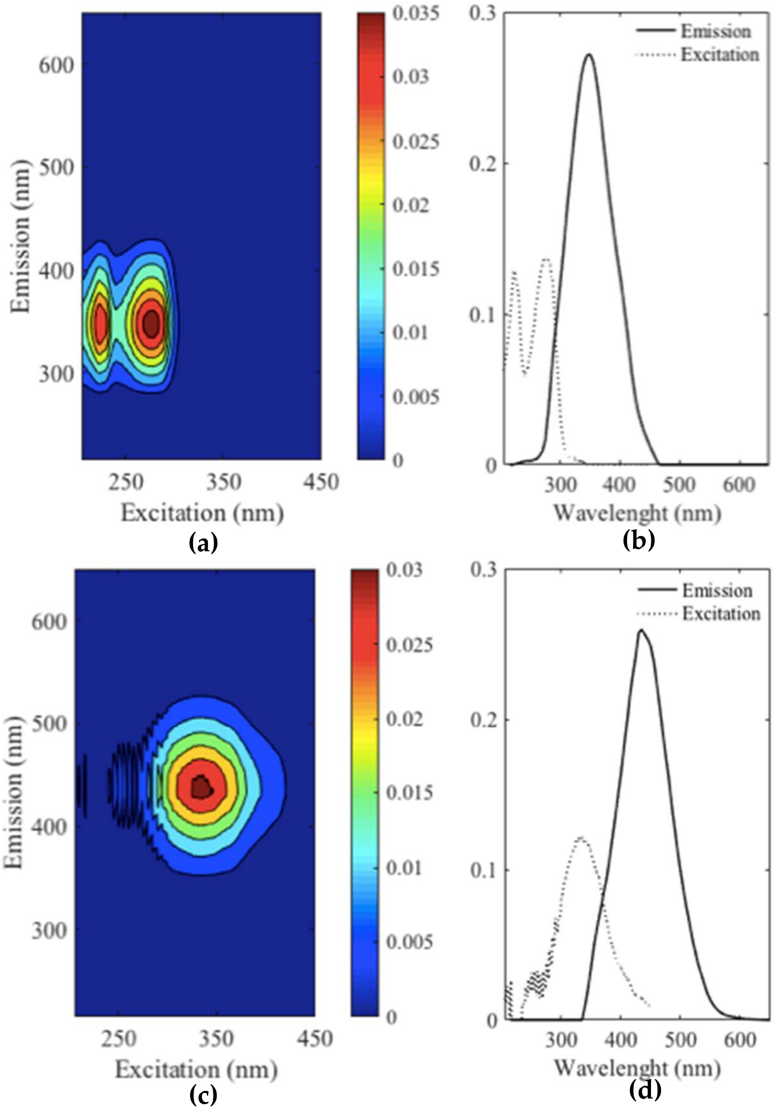
The Gall samples showed lower fluorescence intensities than INV, as well as INVGall, which showed a slight decrease of fluorescence when compared to Gall, and a considerable decrease of intensity when compared to INV (Fig. 2). The reduction in fluorescence (quenching) caused by the addition of Gall could be due to molecular interactions between gallic acid and compounds present in the sparkling wine. Thus, there is an indication of interactions between Gall and INV, as well as interactions between Gall and other molecules present in the control sample.

In order to better understand the interactions between proteins and other compounds present in the wine, the emission following excitation at 278 nm was examined and the results are shown in Table 3. The results showed a decrease in fluorescence intensity (FI) for the addition of Gall and INVGall and an increase in FI for the remaining samples when compared to Ctrl. A small decrease in λ_{em} was observed for all samples, suggesting that the tryptophan present in the proteins of the control interacted with the compounds added resulting in small emission quenching. When observing the emission wavelength and FI for Gall and INVGall, it is noted that the proteins have an excellent affinity to phenols, most likely causing changes in protein conformation that resulted in fluorescence quenching and a small tryptophan blue shift [23].

Table 3. Emission wavelength and the corresponding fluorescence intensity following λ_{ex} 278 nm.

Sample	**λ_{em}**	**FI**
Ctrl	350	52222
Alc	335	75279
INV	335	73024
BSA	340	72225
MAN	340	46462
Gall	340	70930
Asn	335	73377
Trp	345	45953
INVAsn	335	73257

The Parafac model selected had two components showing 65.8% core consistency and was appropriate to explain 95.8% of the data variation. The $\lambda_{ex}/\lambda_{em}$ pattern obtained by the Parafac model is shown in Figure 3. The results obtained by the first component are defined by two regions of $\lambda_{ex}/\lambda_{em}$ = 222-249/330-360 nm and $\lambda_{ex}/\lambda_{em}$ = 274-285/350 nm (Fig. 3a-b); the second component showed one region with maximum $\lambda_{ex}/\lambda_{em}$ = 326-345/435 nm (Fig. 4c-d). The Parafac scores, when compared to foam parameters, did not show a clear correlation (data not shown), thus, principal component analysis (PCA) was applied. Hence, three principal components were required, corresponding to the three regions (emission at 245 nm, 350 nm, and 435 nm). The results for each model and the scores related for each variable for each principal component are described in Table S1 (supplementary data). The PCA did not show any significant relationship for the region of $\lambda_{ex}/\lambda_{em}$ = 222-249/330-360 nm. However, for the $\lambda_{ex}/\lambda_{em}$ = 274-285/350 nm region, a positive relationship with h and a negative relationship with D_r was found. In addition, a positive relationship was found with h_c for the second component (region $\lambda_{ex}/\lambda_{em}$ = 326-345/435 nm).



(a) (b)

(c) (d)

Figure 3. (a) contour plot for component 1 and **(b)** respective loading plot; **(c)** contour plot for component 2 and **(d)** respective loading plot.

4. Discussion

The purpose of this study was to investigate the effect of several compounds, and interactions among proteins, amino acids, and polyphenols on the characteristics of foam in sparkling wines by exploring several hypotheses previously formulated. This session looks at the identification of compounds associated to foam quality, by using fluorescence spectroscopy, and further discusses those hypotheses.

4.1. Identification of compounds related to foam quality

The fingerprint for the samples analyzed by fluorescence spectroscopy was presented by the principal components (Fig. 3a-d). The figure has indicated the presence of several compounds, such as those originated from yeast/bacteria, polyphenols and proteins that were further associated to foam parameters.

The left region of the first component is characterized by a peak of short excitation and short emission wavelength (Fig. 3a-b), suggesting it is related to low molecular mass, simple aromatic compounds. Aromatic amino acids and nucleic acids (AAA+NA) are excited at 250-260 nm and emit fluorescence in the range of 331-336 nm [27,28]. Tryptophans found in the AAA+NA are the compounds that contribute mostly to the fluorescence, although its quantum yields are 100 times lower than quantum yields of tryptophan's molecules [29]. Hence, component 1 could indicate the presence of AAA+NA, perhaps originating from Lctobacillus and/or yeast [27,28]. The pattern expressed by the second region of the first component $\lambda_{ex}/\lambda_{em}$ = 285/350-363 nm is more likely to have resulted from the fluorophores from different polyphenols and proteins. Polyphenols are excited in the range of 260-330 nm and show emission in the range 310-442 nm [30]. Catechin, epicatechin, epigallocatechin and procyanidin, exhibit a pattern $\lambda_{ex}/\lambda_{em}$ = 280-290/310-320 and gallic acid $\lambda_{ex}/\lambda_{em}$ = 278-280/320-366. Nevertheless, proteins also have similar fluorescence patterns ($\lambda_{ex}/\lambda_{em}$ = 279-295/300-350) [23]. The compounds responsible for fluorescence in this region were found to be positively related to $\boldsymbol{h}$, and negatively related to $\boldsymbol{D_r}$. The addition of mannose was found to increase $\boldsymbol{h}$ (Fig. 2), thus, they are the most probable compounds related to the findings of the PCA regarding the first component of the Parafac analysis. Consequently, we can determine that proteins present in the wine matrix do influence foam stability by decreasing the drainability (Fig. 1) and maintaining the presence of a collar for the duration of 300 seconds (definition of $\boldsymbol{h}$ [21]).

The region represented by the second component ($\lambda_{ex}/\lambda_{em}$ = 326-345/435 nm) likely results from the fluorescence of the hydroxycinnamic acids (HCAs) present in the sparkling wines. HCAs have absorption around 325 nm and maximum fluorescence emission at 440 nm [31] and are present naturally in white wines [32] originated from grape pulp [6]. However, they are found in the form of tartaric acid esters highly susceptible to hydrolysis [6].

An unexpected result observed was the significant influence of polyphenols and hydroxycinnamates on foam parameters. Polyphenols, most likely non-flavonoids, seem to affect positively the foam characteristics, most importantly, $\boldsymbol{S_b}$ and $\boldsymbol{h_c}$. This is the first study we could uncover reporting a compound that was able to increase the parameter $\boldsymbol{S_b}$. It is desirable to have a high number of small bubbles in sparkling wines, as to enhance its perceived quality. However, when added simultaneously to INV, phenols might present an opposite effect to bubble size as observed in Fig. 1. A recent study has found important correlations between foamability and stability, and anthocyanins, but did not find any influence of HCAs on foam quality [10]. The discrepancy in the observed results is more likely due to the different methodology applied to measure the foam parameters.

4.2. Hypothesis

Previous investigation on the effect of proteins and amino acids in foam parameters [19] has raised several hypotheses. This study has investigated these specific hypotheses, which are discussed in detail below.

(i) proteins and amino acids influence foam properties, such as foam stability

Our hypothesis testing (i) has shown that indeed proteins and amino acids significantly affect foam properties, such as parameters representative of foam stability ($\boldsymbol{D_r}$, $\boldsymbol{F_v}$, $\boldsymbol{L_f}$). Amino acids might promote foamability but might have a negative effect on foam stability (Fig. 1). Also, different proteins have different compositions resulting in specific interactions to wine compounds, consequently affecting foam quality differently. For instance, BSA forms hydrogen bonds/hydrophobic interactions to proanthocyanidins and catechins originated from grapes and white wines, resulting in an increase of hydrophobicity of the molecules [33]. On the other hand, the different composition and conformation of glycosylated proteins increase the possibility of binding to phenolic compounds and decrease the potential to form and stabilize foams.

Mannoproteins are generally composed of 20% proteins and 80% D-mannose associated with D-glucose [34] and have been found to have a good affinity to flavonols [35]. Yeast invertase, similarly to mannoproteins, have a lower ratio of proteins to glycosylated compounds (mannose/glucose) [36]. The association of MAN and INV to flavonols is more likely to happen between the glycosylated moieties than to the protein side [36]. Although it might be anticipated that INV and MAN would show similar effects on foam parameters, it is worth noting that while INV had a high purity, MAN was a commercial product, which contains other compounds that might impact of how the proteins interact with other wine chemicals.

Hence, we speculate that the significant influence of BSA on L_f (Fig. 1) could be related to an increase in hydrophobicity in proteins (showing similar behavior to BSA) caused by interactions to the phenolic compounds present in the wines, most likely proanthocyanidins, or is promoted by proteins which are hydrophobic. Hydrophobic compounds have more affinity to the gas and other compounds in the bubble walls [37], resulting in a resistant viscoelastic film [2], thus promoting foam stability. On the other hand, the complex formed between the phenolic compounds and glycosylated proteins seems to be detrimental to foam quality.

(ii) compounds interact with proteins resulting in a positive or negative effect on foam quality;

The interaction between proteins and other compounds was suggested by the contour plots (Fig. 2) and become more evident when assessing the FI and emission wavelength shifts that occurred when λ_{ex} is 278 nm. The FI increase observed for BSA, INV, INVAsn, MAN, and Trp (Table 3) is probably due to an increased concentration of tryptophan residues while the increase in FI seen for Alc and Asn (Table 3) is more likely to be caused by structural changes undergone by the proteins present in the wine when exposed to those compounds, resulted from exposure of buried tryptophan residues. The fluorescence quenching observed in the presence of Gall and INVGall highlights the interaction between those compounds, and also, interactions to other compounds present in the wine matrix. Furthermore, the quenching of tryptophan emission is indicative of tryptophan location [38] and changes in protein conformation [23]. The tertiary structure of proteins has been found to change its conformation when binding to phenolic compounds [39]. Tryptophan residues fully exposed emits at 350 nm; partially exposed, at 340 nm; buried within a protein, but interacting to the surrounded environment, at 315-330 nm; and fully buried, at 308 nm. The observed blue shift is probably the result of hydrogen bonds between –OH moieties in polyphenols and the NH_2, OH and SH groups in the protein [39,40]. The proteins found in the foam of sparkling wines are mostly amphiphilic [41]. The electrostatic interaction between proteins and polyphenols may result from the hydrophilic groups from the proteins and OH groups in polyphenols resulting in stable protein-polyphenol interaction. The high affinity of tryptophan residues to the compounds present in the wine analyzed is emphasized by the blue shift in the emission observed for Trp (Table 3). Compounds present in the wine easily interact with the tryptophan residues. We speculate that the polyphenols in the sparkling wine are very likely to interact with tryptophan residues, and thus, have high affinity for proteins. Therefore, changes in the proteins' conformation and stable interactions with polyphenols impair the likelihood of the protein being adsorbed in the foam matrix, consequently influencing negatively foam stability and foam formation. Additionally, the interactions between proteins and amino acids were also showed to impair foam quality (Fig. 1). For instance, the foam promoting effect of Asn was neutralized by INV (Fig.1) and the positive impact of yeast proteins and phenols on bubbles size, observed by an increase of the number of small bubbles ratio as seen in Fig. 2 (Gall and INV increased S_b), was counteracted when both compounds were added simultaneously – a decrease in S_b was observed.

Additionally, it seems that proteins might not occur alone in the wine matrix. Our work suggests that the high affinity of phenolic compounds to the wine proteins means that proteins are likely be found bound to compounds, resulting in a negative effect on foam quality, or a positive effect (such as found in BSA – previously discussed in hypothesis i).

iii) proteins (principally those originated from yeast) and amino acids increase L_f

The results did not support the assumption of yeast proteins and amino acids increasing L_f (iii). The parameter L_f was found to be increased by the presence of BSA. When comparing L_f obtained from the control and INV samples, it is observed that INV decreased L_f. The decrease in L_f observed when adding yeast proteins might be due to strong interactions between INV and other compounds present in the wine sample, such as phenolic compounds, which resulted in the yeast proteins being unable to be absorbed in the film layer, and consequently, unable to provide stability to the foam.

5. Conclusions

The results of the present study have shown that several compounds present in sparkling wines influence foam quality differently, as well as highlighting the importance of the interactions between these compounds and other wine components. Mannoproteins were found to be most likely to promote foam and collar stability, while phenols were likely to increase the number of small bubbles in the foam matrix. Our work confirmed amino acids influence foam quality and showed how different proteins influence different foam parameters. Additionally, our study showed that polyphenols have high affinity to proteins present in sparkling wines, and this interaction might be positive or negative on foam characteristics. In summary, the interactions and the resulting effect on foam parameters in sparkling wines are extremely complex; however, the findings here can be adequately explained, and the techniques used here applied might assist us to better understand the consequences of those interactions and provide us with tools to be able to control and modify sparkling wine foam quality.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Variable scores for each principal component related to the three principal component analysis (λ_{em} 245 nm, λ_{em} 350 nm, λ_{em} 435 nm).

Abbreviations: average foam lifetime (L_f); collar height (h); collar initial height (h_c); drainability (D_r); λ_{ex}, excitation wavelength; λ_{em}, emission wavelength; foam expansion (E); foam time (F_t); foam velocity (F_v); foam volume (V_f); percentage of wine in the foam (W_f); and small bubbles ratio (S_b);

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Conflicts of Interest: "The authors declare no conflict of interest."

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Supplementary material.

Table S1. Variable scores for each principal component related to the three principal component analysis (λ_{em} 245 nm, λ_{em} 350 nm, λ_{em} 435 nm).

Variables	λ_{em} 245 nm			λ_{em} 350 nm			λ_{em} 435		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
V_f	0.08	0.91	0.10	-0.35	0.03	0.23	-0.34	0.11	0.86
h_c	0.05	0.13	-0.45	0.17	0.34	0.33	0.30	0.59	0.15
F_v	0.30	0.93	-0.14	-0.44	-0.07	0.44	-0.40	0.25	0.84
D_r	0.10	0.97	0.02	-0.54	-0.03	0.24	-0.47	0.26	0.82
L_f	-0.20	-0.80	0.07	0.37	0.07	-0.26	0.31	-0.25	-0.69
E	0.06	0.71	0.55	-0.22	0.01	0.16	-0.30	-0.16	0.75
h	0.23	-0.44	0.50	0.46	0.07	0.28	0.40	-0.03	-0.12
S_b	0.09	0.05	0.94	0.05	-0.30	-0.11	-0.02	-0.35	0.26
λ_{ex} 205	0.97	0.09	0.06	0.91	-0.32	-0.23	N/A	N/A	N/A
λ_{ex} 206	0.98	0.03	0.09	0.93	-0.28	-0.23	N/A	N/A	N/A
λ_{ex} 207	0.91	-0.16	0.18	0.93	-0.27	-0.24	N/A	N/A	N/A
λ_{ex} 208	0.96	-0.16	0.06	0.93	-0.26	-0.23	N/A	N/A	N/A
λ_{ex} 209	0.92	-0.19	0.10	0.95	-0.26	-0.16	N/A	N/A	N/A
λ_{ex} 210	0.99	-0.03	0.06	0.95	-0.22	-0.18	N/A	N/A	N/A
λ_{ex} 211	0.97	-0.16	-0.08	0.96	-0.20	-0.18	N/A	N/A	N/A
λ_{ex} 212	0.89	-0.11	-0.18	0.97	-0.17	-0.17	N/A	N/A	N/A
λ_{ex} 213	0.96	-0.24	0.01	0.97	-0.09	-0.17	N/A	N/A	N/A
λ_{ex} 214	0.94	0.10	-0.10	0.98	-0.04	-0.18	N/A	N/A	N/A
λ_{ex} 215	0.98	-0.10	0.05	0.99	-0.01	-0.08	N/A	N/A	N/A
λ_{ex} 216	0.98	-0.10	-0.03	0.98	0.07	-0.12	N/A	N/A	N/A
λ_{ex} 217	0.95	-0.06	-0.07	0.97	0.23	-0.08	N/A	N/A	N/A
λ_{ex} 218	0.95	0.07	-0.15	0.94	0.31	-0.01	N/A	N/A	N/A
λ_{ex} 219	0.97	0.00	-0.01	0.92	0.37	0.03	N/A	N/A	N/A
λ_{ex} 220	0.98	-0.06	-0.13	0.87	0.48	0.06	N/A	N/A	N/A
λ_{ex} 221	1.00	0.00	-0.01	0.80	0.57	0.08	N/A	N/A	N/A
λ_{ex} 222	0.98	-0.10	0.06	0.77	0.60	0.13	N/A	N/A	N/A
λ_{ex} 223	0.99	-0.08	-0.03	0.78	0.59	0.11	N/A	N/A	N/A
λ_{ex} 224	0.97	-0.06	0.00	0.78	0.61	0.06	N/A	N/A	N/A
λ_{ex} 225	0.99	0.00	-0.06	0.79	0.59	0.04	N/A	N/A	N/A
λ_{ex} 226	0.99	0.01	0.11	0.84	0.53	-0.03	N/A	N/A	N/A
λ_{ex} 227	0.99	0.00	0.00	0.88	0.45	-0.06	N/A	N/A	N/A
λ_{ex} 228	0.96	-0.13	0.09	0.91	0.40	-0.06	N/A	N/A	N/A
λ_{ex} 229	0.97	-0.14	-0.04	0.92	0.32	-0.14	N/A	N/A	N/A
λ_{ex} 230	0.99	0.00	0.04	0.93	0.31	-0.15	N/A	N/A	N/A
λ_{ex} 231	0.98	-0.06	0.06	0.96	0.18	-0.17	N/A	N/A	N/A
λ_{ex} 232	0.99	-0.03	-0.01	0.97	0.05	-0.21	N/A	N/A	N/A

λ_{ex} 233	0.99	0.05	-0.01	0.96	-0.11	-0.26	N/A	N/A	N/A
λ_{ex} 234	1.00	0.04	-0.03	0.95	-0.17	-0.26	N/A	N/A	N/A
λ_{ex} 235	0.99	0.06	-0.02	0.93	-0.23	-0.29	N/A	N/A	N/A
λ_{ex} 236	1.00	0.01	-0.01	0.93	-0.24	-0.28	N/A	N/A	N/A
λ_{ex} 237	0.99	0.04	-0.01	0.92	-0.26	-0.28	N/A	N/A	N/A
λ_{ex} 238	0.99	0.01	0.01	0.92	-0.27	-0.28	N/A	N/A	N/A
λ_{ex} 239	0.99	-0.05	0.02	0.92	-0.28	-0.27	N/A	N/A	N/A
λ_{ex} 240	0.92	0.25	-0.06	0.92	-0.27	-0.28	N/A	N/A	N/A
λ_{ex} 241	0.94	0.19	-0.08	0.92	-0.27	-0.28	N/A	N/A	N/A
λ_{ex} 242	0.94	0.16	-0.04	0.92	-0.26	-0.27	N/A	N/A	N/A
λ_{ex} 243	0.95	0.20	-0.03	0.92	-0.26	-0.28	N/A	N/A	N/A
λ_{ex} 244	0.97	0.12	0.00	0.93	-0.26	-0.27	N/A	N/A	N/A
λ_{ex} 245	N/A	N/A	N/A	0.93	-0.23	-0.26	N/A	N/A	N/A
λ_{ex} 246	N/A	N/A	N/A	0.94	-0.23	-0.25	N/A	N/A	N/A
λ_{ex} 247	N/A	N/A	N/A	0.95	-0.18	-0.25	N/A	N/A	N/A
λ_{ex} 248	N/A	N/A	N/A	0.95	-0.16	-0.25	0.95	-0.28	0.04
λ_{ex} 249	N/A	N/A	N/A	0.96	-0.13	-0.22	0.95	-0.29	0.03
λ_{ex} 250	N/A	N/A	N/A	0.97	-0.09	-0.23	0.96	-0.27	0.03
λ_{ex} 251	N/A	N/A	N/A	0.97	-0.08	-0.22	0.94	-0.31	0.03
λ_{ex} 252	N/A	N/A	N/A	0.98	-0.04	-0.17	0.94	-0.30	-0.01
λ_{ex} 253	N/A	N/A	N/A	0.98	0.01	-0.16	0.92	-0.37	0.01
λ_{ex} 254	N/A	N/A	N/A	0.98	0.09	-0.15	0.92	-0.36	0.02
λ_{ex} 255	N/A	N/A	N/A	0.98	0.14	-0.12	0.92	-0.39	0.02
λ_{ex} 256	N/A	N/A	N/A	0.97	0.19	-0.10	0.92	-0.37	0.01
λ_{ex} 257	N/A	N/A	N/A	0.95	0.30	-0.07	0.92	-0.39	0.01
λ_{ex} 258	N/A	N/A	N/A	0.93	0.36	-0.05	0.91	-0.40	0.03
λ_{ex} 259	N/A	N/A	N/A	0.89	0.43	0.04	0.91	-0.41	0.02
λ_{ex} 260	N/A	N/A	N/A	0.87	0.48	0.00	0.91	-0.41	0.01
λ_{ex} 261	N/A	N/A	N/A	0.84	0.53	0.06	0.91	-0.40	0.02
λ_{ex} 262	N/A	N/A	N/A	0.81	0.57	0.10	0.91	-0.40	0.04
λ_{ex} 263	N/A	N/A	N/A	0.80	0.58	0.11	0.89	-0.44	0.01
λ_{ex} 264	N/A	N/A	N/A	0.78	0.61	0.12	0.89	-0.46	0.03
λ_{ex} 265	N/A	N/A	N/A	0.74	0.65	0.14	0.90	-0.43	0.01
λ_{ex} 266	N/A	N/A	N/A	0.70	0.68	0.17	0.89	-0.46	0.00
λ_{ex} 267	N/A	N/A	N/A	0.69	0.68	0.22	0.88	-0.47	0.02
λ_{ex} 268	N/A	N/A	N/A	0.69	0.68	0.26	0.88	-0.48	0.03
λ_{ex} 269	N/A	N/A	N/A	0.68	0.67	0.29	0.88	-0.47	0.00
λ_{ex} 270	N/A	N/A	N/A	0.66	0.65	0.35	0.88	-0.47	0.02
λ_{ex} 271	N/A	N/A	N/A	0.67	0.66	0.35	0.88	-0.46	0.03
λ_{ex} 272	N/A	N/A	N/A	0.67	0.66	0.33	0.89	-0.46	0.02
λ_{ex} 273	N/A	N/A	N/A	0.66	0.65	0.36	0.87	-0.48	0.04
λ_{ex} 274	N/A	N/A	N/A	0.67	0.66	0.35	0.88	-0.47	0.04
λ_{ex} 275	N/A	N/A	N/A	0.66	0.65	0.36	0.87	-0.49	0.01
λ_{ex} 276	N/A	N/A	N/A	0.67	0.65	0.35	0.88	-0.48	0.02
λ_{ex} 277	N/A	N/A	N/A	0.67	0.65	0.35	0.88	-0.46	0.04
λ_{ex} 278	N/A	N/A	N/A	0.66	0.65	0.37	0.88	-0.46	0.02
λ_{ex} 279	N/A	N/A	N/A	0.67	0.66	0.33	0.88	-0.47	0.02

λ_{ex} 280	N/A	N/A	N/A	0.67	0.66	0.33	0.89	-0.46	0.01
λ_{ex} 281	N/A	N/A	N/A	0.67	0.66	0.34	0.88	-0.48	0.02
λ_{ex} 282	N/A	N/A	N/A	0.67	0.66	0.32	0.89	-0.46	0.02
λ_{ex} 283	N/A	N/A	N/A	0.68	0.66	0.32	0.89	-0.45	0.02
λ_{ex} 284	N/A	N/A	N/A	0.68	0.67	0.29	0.88	-0.47	0.02
λ_{ex} 285	N/A	N/A	N/A	0.69	0.68	0.24	0.89	-0.46	0.04
λ_{ex} 286	N/A	N/A	N/A	0.69	0.68	0.23	0.89	-0.45	0.00
λ_{ex} 287	N/A	N/A	N/A	0.71	0.68	0.16	0.90	-0.43	0.00
λ_{ex} 288	N/A	N/A	N/A	0.74	0.66	0.13	0.90	-0.43	0.05
λ_{ex} 289	N/A	N/A	N/A	0.80	0.58	0.10	0.89	-0.45	0.03
λ_{ex} 290	N/A	N/A	N/A	0.88	0.46	0.01	0.89	-0.45	0.02
λ_{ex} 291	N/A	N/A	N/A	0.95	0.30	-0.06	0.89	-0.45	0.02
λ_{ex} 292	N/A	N/A	N/A	0.98	0.15	-0.12	0.91	-0.42	0.01
λ_{ex} 293	N/A	N/A	N/A	0.98	0.01	-0.16	0.88	-0.46	0.05
λ_{ex} 294	N/A	N/A	N/A	0.97	-0.09	-0.18	0.90	-0.43	0.01
λ_{ex} 295	N/A	N/A	N/A	0.96	-0.17	-0.21	0.91	-0.40	0.05
λ_{ex} 296	N/A	N/A	N/A	0.94	-0.20	-0.23	0.90	-0.43	0.03
λ_{ex} 297	N/A	N/A	N/A	0.93	-0.24	-0.24	0.91	-0.39	0.03
λ_{ex} 298	N/A	N/A	N/A	0.93	-0.24	-0.24	0.90	-0.42	0.02
λ_{ex} 299	N/A	N/A	N/A	0.93	-0.24	-0.23	0.91	-0.40	0.03
λ_{ex} 300	N/A	N/A	N/A	0.93	-0.24	-0.24	0.93	-0.36	0.03
λ_{ex} 301	N/A	N/A	N/A	0.94	-0.24	-0.23	0.92	-0.36	0.04
λ_{ex} 302	N/A	N/A	N/A	0.94	-0.23	-0.23	0.93	-0.33	0.00
λ_{ex} 303	N/A	N/A	N/A	0.95	-0.20	-0.22	0.92	-0.31	0.05
λ_{ex} 304	N/A	N/A	N/A	0.95	-0.22	-0.20	0.93	-0.29	0.05
λ_{ex} 305	N/A	N/A	N/A	0.95	-0.20	-0.22	0.93	-0.24	0.05
λ_{ex} 306	N/A	N/A	N/A	0.96	-0.20	-0.20	0.94	-0.25	0.05
λ_{ex} 307	N/A	N/A	N/A	0.96	-0.18	-0.20	0.94	-0.18	-0.02
λ_{ex} 308	N/A	N/A	N/A	0.96	-0.20	-0.17	0.94	-0.23	0.01
λ_{ex} 309	N/A	N/A	N/A	0.97	-0.17	-0.15	0.95	-0.24	0.00
λ_{ex} 310	N/A	N/A	N/A	0.97	-0.19	-0.12	0.95	-0.15	0.04
λ_{ex} 311	N/A	N/A	N/A	0.96	-0.15	-0.14	0.96	-0.19	0.00
λ_{ex} 312	N/A	N/A	N/A	0.95	-0.17	-0.09	0.97	-0.18	0.00
λ_{ex} 313	N/A	N/A	N/A	0.97	-0.17	-0.06	0.98	-0.09	-0.02
λ_{ex} 314	N/A	N/A	N/A	0.93	-0.23	-0.02	0.97	-0.17	-0.01
λ_{ex} 315	N/A	N/A	N/A	0.94	-0.22	0.00	0.97	-0.07	0.00
λ_{ex} 316	N/A	N/A	N/A	0.94	-0.25	0.10	0.98	-0.10	0.00
λ_{ex} 317	N/A	N/A	N/A	0.92	-0.32	0.07	0.98	0.01	0.00
λ_{ex} 318	N/A	N/A	N/A	0.89	-0.34	0.04	0.98	-0.01	0.01
λ_{ex} 319	N/A	N/A	N/A	0.86	-0.43	0.10	0.98	0.08	-0.02
λ_{ex} 320	N/A	N/A	N/A	0.88	-0.46	0.04	0.98	0.09	0.05
λ_{ex} 321	N/A	N/A	N/A	0.92	-0.38	0.04	0.97	0.11	-0.06
λ_{ex} 322	N/A	N/A	N/A	0.88	-0.44	0.02	0.95	0.25	-0.03
λ_{ex} 323	N/A	N/A	N/A	0.89	-0.42	0.13	0.93	0.31	0.00
λ_{ex} 324	N/A	N/A	N/A	0.84	-0.46	0.20	0.94	0.29	-0.06
λ_{ex} 325	N/A	N/A	N/A	0.83	-0.52	0.15	0.90	0.41	-0.08
λ_{ex} 326	N/A	N/A	N/A	0.80	-0.57	0.16	0.88	0.42	-0.11

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λ_{ex} 327	N/A	N/A	N/A	0.80	-0.55	0.19	0.88	0.41	-0.15
λ_{ex} 328	N/A	N/A	N/A	0.78	-0.55	0.28	0.59	0.60	0.02
λ_{ex} 329	N/A	N/A	N/A	0.78	-0.53	0.29	0.72	0.61	0.11
λ_{ex} 330	N/A	N/A	N/A	0.73	-0.55	0.37	0.66	0.59	0.10
λ_{ex} 331	N/A	N/A	N/A	0.74	-0.58	0.33	0.60	0.73	0.01
λ_{ex} 332	N/A	N/A	N/A	0.77	-0.52	0.36	0.66	0.73	-0.01
λ_{ex} 333	N/A	N/A	N/A	0.68	-0.60	0.42	0.67	0.71	0.09
λ_{ex} 334	N/A	N/A	N/A	0.69	-0.61	0.37	0.74	0.65	0.12
λ_{ex} 335	N/A	N/A	N/A	0.67	-0.58	0.45	0.70	0.67	0.12
λ_{ex} 336	N/A	N/A	N/A	0.67	-0.58	0.45	0.70	0.67	0.14
λ_{ex} 337	N/A	N/A	N/A	0.62	-0.60	0.49	0.59	0.80	0.00
λ_{ex} 338	N/A	N/A	N/A	0.63	-0.56	0.53	0.66	0.72	0.05
λ_{ex} 339	N/A	N/A	N/A	0.59	-0.60	0.52	0.61	0.76	-0.03
λ_{ex} 340	N/A	N/A	N/A	0.56	-0.60	0.56	0.64	0.76	-0.02
λ_{ex} 341	N/A	N/A	N/A	0.56	-0.59	0.57	0.73	0.64	-0.01
λ_{ex} 342	N/A	N/A	N/A	0.53	-0.62	0.58	0.78	0.60	0.00
λ_{ex} 343	N/A	N/A	N/A	0.49	-0.59	0.63	0.77	0.61	-0.05
λ_{ex} 344	N/A	N/A	N/A	0.51	-0.62	0.58	0.80	0.59	-0.02
λ_{ex} 345	N/A	N/A	N/A	0.42	-0.6	0.66	0.83	0.53	-0.04
λ_{ex} 346	N/A	N/A	N/A	0.35	-0.58	0.71	0.76	0.60	-0.03
λ_{ex} 347	N/A	N/A	N/A	0.38	-0.57	0.71	0.79	0.56	0.03
λ_{ex} 348	N/A	N/A	N/A	0.30	-0.58	0.73	0.71	0.69	-0.05
λ_{ex} 349	N/A	N/A	N/A	0.26	-0.55	0.78	0.80	0.56	-0.07
λ_{ex} 350	N/A	N/A	N/A	0.99	0.68	0.78	0.75	0.64	-0.10
λ_{ex} 351	N/A	N/A	N/A	N/A	N/A	N/A	0.81	0.54	0.01
λ_{ex} 352	N/A	N/A	N/A	N/A	N/A	N/A	0.79	0.56	-0.05
λ_{ex} 353	N/A	N/A	N/A	N/A	N/A	N/A	0.76	0.61	0.05
λ_{ex} 354	N/A	N/A	N/A	N/A	N/A	N/A	0.87	0.47	-0.09
λ_{ex} 355	N/A	N/A	N/A	N/A	N/A	N/A	0.81	0.57	-0.04
λ_{ex} 356	N/A	N/A	N/A	N/A	N/A	N/A	0.88	0.46	-0.01
λ_{ex} 357	N/A	N/A	N/A	N/A	N/A	N/A	0.87	0.47	-0.02
λ_{ex} 358	N/A	N/A	N/A	N/A	N/A	N/A	0.79	0.57	-0.02
λ_{ex} 359	N/A	N/A	N/A	N/A	N/A	N/A	0.81	0.51	-0.09
λ_{ex} 360	N/A	N/A	N/A	N/A	N/A	N/A	0.86	0.50	0.05
λ_{ex} 361	N/A	N/A	N/A	N/A	N/A	N/A	0.95	0.30	0.00
λ_{ex} 362	N/A	N/A	N/A	N/A	N/A	N/A	0.94	0.31	-0.02
λ_{ex} 363	N/A	N/A	N/A	N/A	N/A	N/A	0.87	0.46	-0.02
λ_{ex} 364	N/A	N/A	N/A	N/A	N/A	N/A	0.91	0.35	-0.15
λ_{ex} 365	N/A	N/A	N/A	N/A	N/A	N/A	0.92	0.30	-0.03
λ_{ex} 366	N/A	N/A	N/A	N/A	N/A	N/A	0.87	0.45	-0.03
λ_{ex} 367	N/A	N/A	N/A	N/A	N/A	N/A	0.92	0.31	-0.09
λ_{ex} 368	N/A	N/A	N/A	N/A	N/A	N/A	0.94	0.33	0.01
λ_{ex} 369	N/A	N/A	N/A	N/A	N/A	N/A	0.89	0.39	-0.09
λ_{ex} 370	N/A	N/A	N/A	N/A	N/A	N/A	0.88	0.42	0.04
λ_{ex} 371	N/A	N/A	N/A	N/A	N/A	N/A	0.95	0.26	0.00
λ_{ex} 372	N/A	N/A	N/A	N/A	N/A	N/A	0.94	0.27	-0.02
λ_{ex} 373	N/A	N/A	N/A	N/A	N/A	N/A	0.96	0.25	0.02

λ_{ex} 374	N/A	N/A	N/A	N/A	N/A	N/A	0.91	0.36	0.06
λ_{ex} 375	N/A	N/A	N/A	N/A	N/A	N/A	0.94	0.28	0.10
λ_{ex} 376	N/A	N/A	N/A	N/A	N/A	N/A	0.92	0.28	0.02
λ_{ex} 377	N/A	N/A	N/A	N/A	N/A	N/A	0.95	0.18	0.04
λ_{ex} 378	N/A	N/A	N/A	N/A	N/A	N/A	0.92	0.30	0.15
λ_{ex} 379	N/A	N/A	N/A	N/A	N/A	N/A	0.95	0.26	0.03
λ_{ex} 380	N/A	N/A	N/A	N/A	N/A	N/A	0.98	0.12	-0.01
λ_{ex} 381	N/A	N/A	N/A	N/A	N/A	N/A	0.98	0.12	0.06
λ_{ex} 382	N/A	N/A	N/A	N/A	N/A	N/A	0.97	0.14	0.09
λ_{ex} 383	N/A	N/A	N/A	N/A	N/A	N/A	0.98	0.08	0.08
λ_{ex} 384	N/A	N/A	N/A	N/A	N/A	N/A	0.98	0.02	0.07
λ_{ex} 385	N/A	N/A	N/A	N/A	N/A	N/A	0.98	0.06	0.09
λ_{ex} 386	N/A	N/A	N/A	N/A	N/A	N/A	0.97	0.12	0.03
λ_{ex} 387	N/A	N/A	N/A	N/A	N/A	N/A	0.97	0.09	0.01
λ_{ex} 388	N/A	N/A	N/A	N/A	N/A	N/A	0.92	0.12	-0.01
λ_{ex} 389	N/A	N/A	N/A	N/A	N/A	N/A	0.95	0.03	-0.03
λ_{ex} 390	N/A	N/A	N/A	N/A	N/A	N/A	0.96	0.03	0.09
λ_{ex} 391	N/A	N/A	N/A	N/A	N/A	N/A	0.96	0.02	0.02
λ_{ex} 392	N/A	N/A	N/A	N/A	N/A	N/A	0.96	-0.05	0.02
Data explanation (%)	**78.8**	**9.4**	**3.9**	**68.0**	**18.8**	**7.9**	**75.1**	**17.5**	**2.4**