



Food Science and Applied Biotechnology

e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
<https://doi.org/10.30721/fsab2021.v4.i2>



Research Article

Reduction of food histamine in white wine by Bromelain, Quercetin and Ascorbic acid

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Abstract

The scope of this work was to reduce the level of the biogenic amine 'histamine' in White wine. Different concentrations of 100 ml wine samples were prepared by incorporating various concentrations Bromelain; Quercetin and Ascorbic acid. A spectrophotometric based histamine analysis method was carried out to determine histamine concentrations. The histamine concentration reduced in 200mg/L Bromelain, 50mg/L Quercetin and 400mg/L Ascorbic acid treated samples by 62.34%, 61.64% and 46.41% respectively. The percentage DPPH scavenging activity was found to be 83.93%, 93.54%, 133.08% respectively against normal wine 86.93%. No significant changes observed in physicochemical properties of wine like functional groups; particle size analysis; effect on amino acids; turbidity; colour analysis; pH and sensory analysis in bromelain treated wine samples as compared to other samples. Reduction of histamine can be carried out by Bromelain (200mg/L) effectively than Quercetin and Ascorbic acid; based on the reducing capacity and the sensory parameters.

Keywords: allergens, ascorbic acid, bromelain, histamine, quercetin

Abbreviations:

BA – Biogenic amine

DPPH – Diphenyl -1- picrylhydrazyl

TLC – Thin layer chromatography

ANOVA – Analysis of variance

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Article history:

Received 16 April 2021

Reviewed 14 May 2021

Accepted 16 June 2021

Available on-line 10 October 2021

<https://doi.org/10.30721/fsab2021.v4.i2.143>

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Introduction

Fermented foods and beverages have been consumed since antiquity and they have been part of diets since then. Later, when industrialization took place, people started using the fermentation technique for stupendous sensory appeal of foods and beverages. Hence, in this way came the emergence of fermented wines, cheeses, fermented dairy products, pickles, baked goods etc. (Adams and Moss 2007).

In wine making, the reducing sugars, fructose and glucose constitute the main sugars of grape pulp. During fermentation, yeast enzymes convert these sugars into ethanol and carbon dioxide in approximately equal proportions with the generation of heat. Additionally, tiny amounts of other products are also formed during the fermentation process. After the primary fermentation is done, the Secondary or Malolactic fermentation takes place using the *Lactobacillus* bacteria which gives the wine a 'buttery' taste (diacetyl is produced). During this fermentation process, the lactic acid bacteria convert malic acid into lactic acid through a direct decarboxylation performed by an enzyme known as malate decarboxylase or the malolactic enzyme. Although, other substrates like amino acids may be metabolized to produce biogenic amines (Grainger and Tattersall 2007). These kinds of compounds, produced by free amino acid decarboxylation, can negatively affect the wine quality and its presence might be considered a possible health risks for some consumers (Henríquez-Aedo et al. 2016). The biogenic amines produced in food by the microbial decarboxylation of amino acids can result in consumers suffering allergic reactions, portrayed by trouble in breathing, itching, rash, regurgitating, fever, and hypertension (Naila et al. 2010).

From a toxicological point of view, histamine is a prominent vaso-active amine causing chemical intoxication, urticaria, abdominal cramps, hypotension, headache, and other health problems (Fan et al. 2015).

It is observed that it is difficult to reduce histamine in a fermented food system, since its amount keeps on increasing throughout the fermentation process (Lee et al. 2018). The emerging methods to reduce BA include modified atmosphere packaging (MAP), irradiation, high hydrostatic pressure

(HHP), microbial modeling and addition of food additives and preservatives (Naila et al. 2010). Although, these methods are expensive. Hence, there is need to develop an economic, sustainable process for reduction of histamine in fermented products.

The use of herbs for treating dermatological problems has been done since historical times. Turmeric, Licorice root, Bromelain, Willow bark, Witch hazel, Chamomile, Yarrow, Oak bark, Aloe vera, Capsaicin, Essential fatty acids, Bioflavonoids like Quercetin and Hesperidin, were used as anti-inflammatory agents for skin diseases. (Lett 2000). The main pineapple enzyme- Bromelain is known to have a high value in the field of health care and therapeutics. It is a natural, non-toxic and better alternative to various chemicals and artificially synthesized medicines (Sahoo and Das 2017). Vitamin C is also known to have anti-inflammatory properties. In one of the study involving 400 people, it has been proved that people with low plasma ascorbate levels have high histamine levels and vice-versa (Clemetson 2018).

In this study, the use of the natural components- Bromelain, Quercetin and Ascorbic acid is done to reduce histamine in white wine.

Materials and Methods

The wine samples were purchased from the local market. The brand was - Samara White Wine (Alcohol content: 11% v/v) manufactured by Sula Vineyards Pvt. Ltd., Nashik, Maharashtra, India. The natural components- Bromelain, Quercetin, Ascorbic acid and Histamine (standard) were purchased from Sisco Research Laboratories Pvt Ltd with other consumables.

Sample Preparation

The sample bottles were maintained with 100mL of wine, to which bromelain of 5 mg, 10 mg, 150 mg/L, 200 mg/L and 250 mg/L were weighed carefully and incorporated into each sample bottle. In similar way, samples of quercetin of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L were made. Likewise, samples of ascorbic acid of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L and 500 mg/L were maintained. These bottles were preserved in refrigerator throughout the process of analysis.

According to the [International Code of Oenological Practices \(2021 issue\)](#), the maximum acceptable limit of ascorbic acid is 300 mg/L although ascorbic acid is class 1 preservative hence it can be considered as GMP level.

Histamine detection

The histamine content was analyzed of each sample by spectrophotometric method at 496 nm as mentioned by Patange, Mukundan, & Kumar (2005). The method was slightly modified to handle liquid samples. A standard graph/curve was made with the help of standard dilutions which gave a reference color scale. Concentration of histamine in wine was determined using this standard curve. The reagent p-phenyldiazonium sulfonate was prepared as per [Koessler and Hanke \(1919\)](#) with negligible modifications. The histamine assay and the wine sample preparation step were performed similar to as described by [Patange et al. \(2005\)](#) ([A. IYER, V. MISHAL 2018](#)).

Turbidity

Turbidity test was performed for Normal white wine as well as for all the concentrations of wine samples i.e. the ones incorporated with Bromelain, Quercetin and Ascorbic acid. For this purpose, the Colorimeter – CL 157 from ELICO was used. ([Charalambous 1984](#)).

Colour analysis

The colour was analysed by software Colour Analysis with (Lab Tools) by Samsung J7 Prime 13 MP lens. The readings were taken in triplicates and then put into the formula of ΔE ([Clydesdale and Ahmed 1978](#)).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

pH

To compare the pH of all the component added wine samples with the normal (pure) white wine; the pH Meter from Toshniwal Inst. Mfg. Pvt. Ltd. was used ([Licht 1985](#)).

Effect on antioxidant property of wine

DPPH (2,2 – Diphenyl -1- picrylhydrazyl) assay was carried out to observe the antioxidant property of the incorporated wine samples. The result was

depicted as the percentage of DPPH scavenging activity by the formula given by [Mishra et al. \(2012\)](#):

$$\% \text{ DPPH SCAVENGING ACTIVITY} = \frac{\text{Absorbance of control sample} - \text{Absorbance of test sample (w.r.t Ascorbic acid)}}{\text{Absorbance of control sample}} \times 100 \quad (2)$$

Effect on amino acids (TLC)

For performing TLC of the wine samples, four TLC glass plates were prepared as given by [Naguib et al. \(1995\)](#) with slight changes. The Retention factor or Rf value was calculated as per below formula:

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}} \quad (3)$$

Sensory analysis

Organoleptic based 9-point hedonic scale sensory analysis was carried out from 10 non-trained panelists for acceptability of wine samples ([Kulkarni et al. 2020](#)). Hedonic panel analyzed taste, flavor, color, sweetness, mouthfeel, aroma and overall acceptability. 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely.

Effect on functional groups (FTIR)

Fourier Transform Infrared Spectroscopy was carried out to know the changes in the functional groups of the two wine samples i.e. the normal wine and the 200 mg/L bromelain incorporated wine. This was done using the Fourier Transform Infrared Spectroscopy- Bruker/Vertex 80 instrument ([Chandra et al. 2021](#)).

Particle size analysis

The particle size analysis of the normal white wine and the best wine sample i.e. Bromelain (200 mg/L) incorporated wine; was carried out using the Particle Size Analyzer- Zeta Sizer – (Malvern) ([Chandra et al. 2021](#)).

Statistical and comparative analysis

One-Way ANOVA was carried out with the software SPSS version 25. Duncan's multiple range test was performed to analyze the data and investigate the significant differences ($p < 0.05$).

Results and Discussion

Histamine concentration in wines

The concentration of histamine in all the incorporated wine samples as compared with the normal wine samples is depicted in Figure 1.

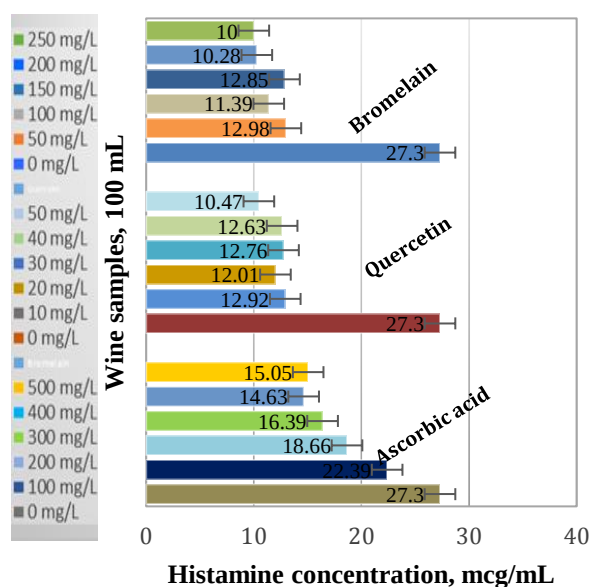


Figure 1. Concentration of Histamine in all the incorporated 100mL wine samples as compared with the normal wine sample.

Turbidity

No significant difference is observed in the wine turbidity of the different bromelain and ascorbic acid concentrations when compared with the normal wine sample (Figure 2). A slight increase in the turbidity was observed for the quercetin samples as the concentration of quercetin increased. This was due to the fact that quercetin was insoluble in nature.

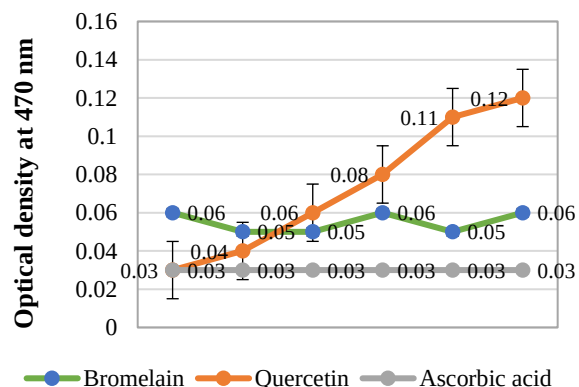


Figure 2. Turbidity of 100 mL wine samples with added Bromelain (50,100,150,200,250 mg/L); Quercetin (10,20,30,40,50 mg/L) and Ascorbic acid (100,200,300,400,500 mg/L) as compared with the normal wine sample (0mg).

Colour analysis

No significant difference is observed in the wine color, of the different bromelain and ascorbic acid concentrations (Figure 2). A significant difference is observed in the wine color, of the different Quercetin concentrations. At the higher concentrations, the ΔE value is immensely high (Figure 3). This was because of the yellow color of quercetin; which gave the wine a yellow appeal.

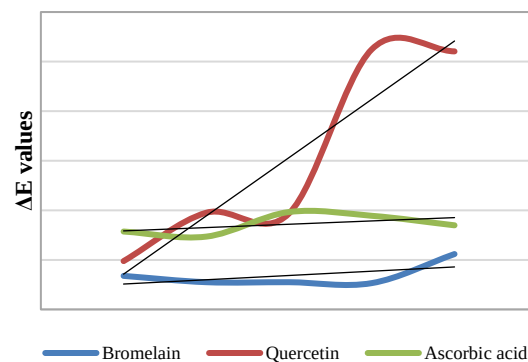


Figure 3. ΔE values of 100 mL wine samples with added Bromelain (50,100,150,200,250 mg/L); Quercetin (10,20,30,40,50 mg/L) and Ascorbic acid (100,200,300,400,500 mg/L).

pH

No significant difference is observed in the wine pH values, of the different bromelain, quercetin and ascorbic acid concentrations as compared to the pH of normal wine sample (pH=3.03).

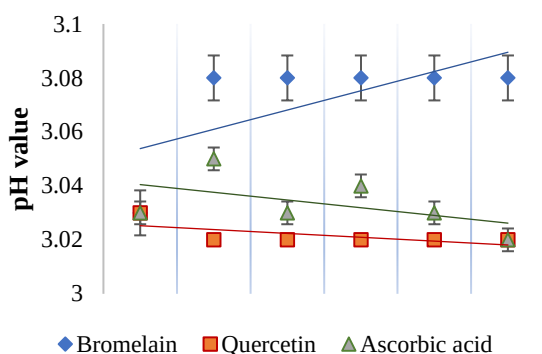


Figure 4. pH values of all the 100 mL wine samples with added Bromelain (50,100,150,200,250 mg/L); Quercetin (10,20,30,40,50 mg/L) and Ascorbic acid (100,200,300,400,500 mg/L) as compared with the normal wine of pH 3.03.

Antioxidant property of wine

The percentage DPPH activity of the wine samples of normal wine, Bromelain (20 mg) wine, Quercetin (50 mg/L) wine and Ascorbic acid (400 mg/L) (undiluted) wine was found to be 86.93%, 83.86%, 93.54% and 133.08% respectively. Figure 5 indicates that there is high antioxidant activity of 400 mg/L ascorbic acid incorporated wine sample as it is a natural antioxidant (Frei et al. 1989). Moreover, Quercetin is also known to have high antioxidant properties (Anand David et al. 2016). Whereas, the 200 mg/L bromelain wine sample showed reduction in the percentage DPPH scavenging activity.

Effect on amino acids (TLC)

The probable amino acid found in all the wine samples are alanine, valine, isoleucine/methionine and norleucine as compared with the (TLC of Aminoacids and Short Peptides, n.d.) as no change in the R_f values was observed. These amino acids give a specific odour to the wines. Alanine gives rosy, honeylike flowery odour; valine depicts an alcoholic fruity odour and isoleucine gives a fruit

odour too. Whereas, methionine is catabolized to release methanethiol which is further converted to sulphur compounds (Styger et al., 2011). Therefore, the TLC results portray no significant difference among the wines. No major changes in the flavour compounds i.e. amino acids has occurred in the wine samples after addition of the natural components.

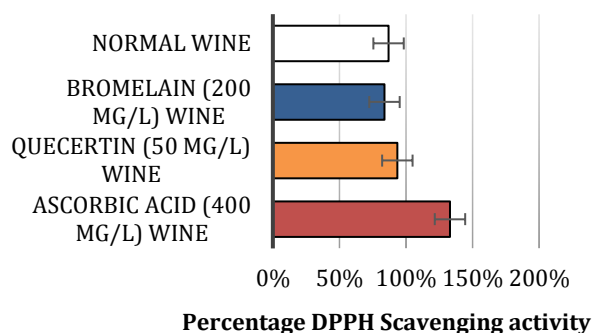


Figure 5. The percentage DPPH Scavenging activity of Bromelain (200 mg/L); Quercetin (50 mg/L) and Ascorbic acid (400 mg/L) incorporated wines as compared with the normal wine sample.

Sensory analysis

The radar graph of the sensory analysis (Figure 6) is represented as dependent on the 9-point hedonic scale values. According to the panelists the normal white wine and the bromelain (200 mg/L) wine samples were found to be similar. The ascorbic acid (400 mg/L) incorporated wine gave a bitter aftertaste and hence it was rejected. So, its concentration needs to be optimized with respect to the organoleptic parameters. Whereas, the quercetin (50 mg/L) incorporated wine sample was also rejected as it led to a drastic change in the color of white wine to yellow. Thus, this wine sample was not greatly accepted by the panelists and rejected based on the color unacceptability. Hence, 200 mg/L bromelain incorporated wine sample was chosen for the further analytical considerations.

In Figure 1, it was observed that the reduction of histamine in the 200 mg/L Bromelain and 50 mg/L Quercetin wines was not significantly different. Whereas, 200 mg/L Bromelain and 400 mg/L Ascorbic acid wines showed a significant difference in the histamine concentrations. Since, quercetin sample was rejected due to yellow color and

ascorbic acid sample was rejected due to its bitter aftertaste. Also, the 50 mg/L quercetin sample showed a significant difference in histamine concentration as compared with the 200 mg/L bromelain wine; the 200 mg/L bromelain wine sample was considered as an efficient and an effective component for the reduction of histamine and for the further analytical considerations.

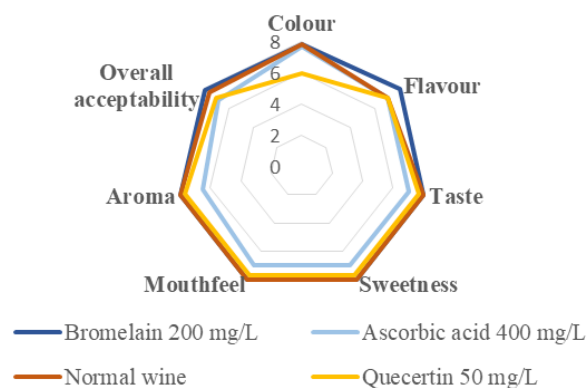


Figure 6. Nine-point hedonic scale spider chart for sensory analysis of wine samples- Normal wine, Bromelain (200mg/L) wine, Quercetin (50mg/L) wine and Ascorbic acid (400mg/L) wine.

Effect on functional groups (FTIR)

As optimised reduction of histamine was observed at bromelain (200mg/L) samples effectively than Quercetin and Ascorbic acid hence the respective analysis considered for FTIR analysis. When compared with the 'Infrared Group Frequencies classification table' (Elena et al. 2012), the compounds alcohols, aldehydes, amines, sulphates and esters were present in both of the samples of wine i.e. normal wine and Bromelain (200mg/L) incorporated wine; which indicates that there is no significant change in the composition of wine functional groups (Figure 7a and 7b). The stretch 3280-3340cm⁻¹ represent the amino acid flavour profile. The flavour profile band stretch remains significantly unchanged but As compared to the normal wine FTIR results, an additional peak at 2719.63 cm⁻¹ was observed in the Bromelain (200mg/L) incorporated wine pertaining to the presence of aldehydes. Although, no significant effect on sensory was found (Devakate et al. 2009)

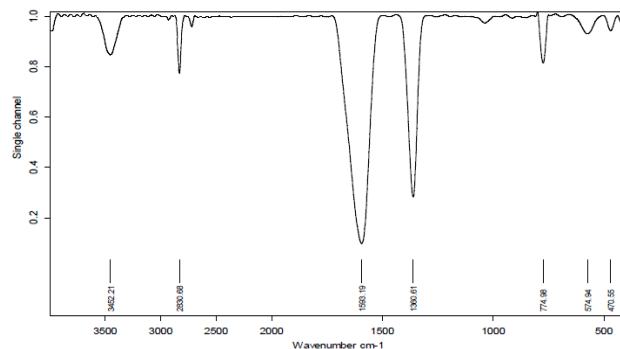


Figure 7(a). FTIR result of Normal wine.

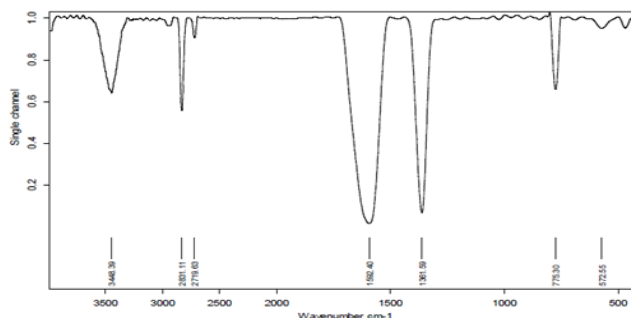


Figure 7(b). FTIR result of Bromelain (200 mg/L) incorporated wine.

Particle size analysis

As optimised reduction of histamine was observed at bromelain (200mg/L) samples effectively than Quercetin and Ascorbic acid hence the respective analysis considered for Particle size analysis.

From the Figure 8a and 8b it is observed that, the Z-Average size of the Bromelain (200mg/L) incorporated white wine sample was found to be 7289 d.nm. Only a single peak was observed of 100% intensity of size 220 d.nm. A significant difference is observed among the two wine samples. The results show that, after the addition of Bromelain of concentration 200 mg/L in wine, the intensity of the size 220 d.nm increases double fold as compared with the normal white wine results. Whereas, all the other four peaks which were observed in the normal wine results are absent in the results of Bromelain wine.

Bromelain is of being an enzyme, it may have led to the agglomeration due to wine pH and proteolysis of molecules present as wine components (Murachi et al. 1964) (Devakate et al. 2009).

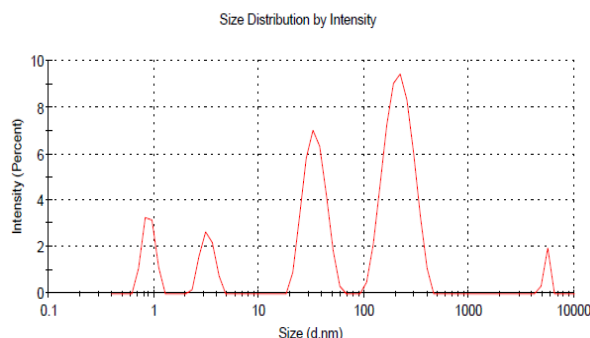


Figure 8(a). Particle size analysis of Normal wine.

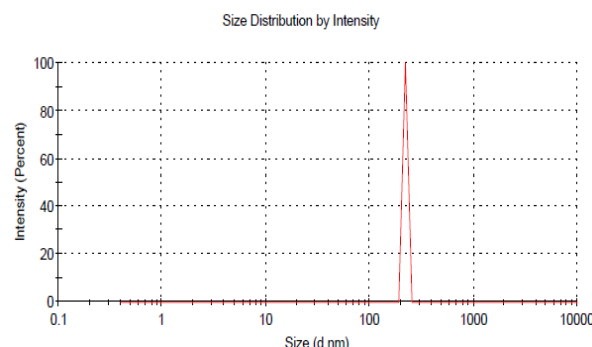


Figure 8(b). Particle size analysis of Bromelain (200 mg/L) incorporated wine.

Conclusions

The abdominal acidity and allergy is major concern for food safety point of view. The wine and fermented products has high percentage of histamine which is causative agent for allergy. The reduction of such histamine component by efficient way with incorporation of natural components like bromelain, which shall not change the physicochemical and sensory attribute of wine as wine, is susceptible to sensory values. The same concept can be use for various fermented products processing where histamine allergen hazard will be a concern for food safety.

Acknowledgements:

A special thanks to Diya Lab (Scientific & Reference Laboratories) for obtaining FTIR and Particle Size Analysis results.

Declaration of interest of statement:

No Conflict of interest present in the given manuscript.

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