INFLUENCE OF THERMOSONICATION ON COLOUR AND CONTENT OF PHENOLIC COMPOUNDS IN RED WINE

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Introduction

Brettanomyces bruxellensis yeast represents a serious problem in winemaking because it can cause off-odours and mousy off-flavours in red wine, as well as high levels of acetic acid and undesirable colour of wine (1). This can lead to significant reduction of wine quality and economic loss for winemakers. Until now, several different approaches were applied in preventing and controlling *B. bruxellensis* presence in winemaking (2). One of new approaches is applying non-thermal processing techniques such as high power ultrasound (HPU). HPU induces mechanical, physical and chemical/biochemical changes through cavitation (3). Combining this treatment with mild temperature (thermosonication) could increase microbial inactivation (4) as well as reduce HPU treatment duration, which could prevent the loss of organoleptic qualities of wine such as colour, flavour and taste. The main compounds in red wine that affect organoleptic qualities and also contribute to the complexity and stability of the wine are phenolics (5). Phenolic compounds are also natural antioxidants that could protect human health and it is important to preserve them in wine (6). The aim of this work was to investigate the impact of thermosonication on colour and phenolic compounds content in red wine during its application for inactivation of *B. bruxellensis* yeast.

Material and Methods

Wine parameters

The wine used for this study was young red wine Cabernet Sauvignon (vintage 2016) obtained from winery Erdutski vinogradi d.o.o., Erdut. The average physico-chemical parameters of the basic young red Cabernet Sauvignon wine were: alcohol 13 % (v/v), pH 3.5, reducing sugars 3.7 g/L, total acidity 6.4 g/L as tartaric acid and volatile acidity 0.6 g/L as acetic acid. pH of wine was adjusted to 3.7 or 3.9 using 10 M NaOH solution. Also, alcohol of wine was corrected to 14 % (v/v) using absolute ethanol, while wine with excess sugar was obtained by 4 g/L glucose addition.

Thermosonic treatment

An amount of 200 mL of different red wine samples, inoculated with *Brettanomyces bruxellensis* CBS 2499 (Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands), were placed in 250 mL round-bottom glass vessels. Before the thermosonic treatment, samples were heated in water bath for 2 min at 43 °C. The samples were then treated by ultrasonic processor (S-4000, Misonix Sonicators, Newtown, CT, USA), set at nominal power of 600 W and constant frequency of 20 kHz with a 12.7 mm diameter probe for 3 min also at 43 °C. The probe was immersed in wine (2.5 cm) and placed at the centre of the round-bottom glass vessel. Ultrasonication was carried out at the amplitude of 120 µm. Each treatment was conducted in triplicate. After treatment, samples were placed in sterile bottles and stored for 90 days at 20 ± 2 °C. Samples were analysed after treatment and after storage time of 90 days.

Colour measurements

The chromatic characteristics were determined using the CIELab (7). The spectra were registered directly on the wine after removing *B. bruxellensis* cells. Colour was expressed as CIE coordinates of L* (lightness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma) and H* (hue angle).

Total phenolics and total anthocyanins measurements

Total phenolics content was determined using the Folin-Ciocalteu method (8) and total anthocyanins content was determined using the SO_2 bleaching method (9) after removing *B. bruxellensis* cells from wine for both analyses. The results of total phenolics were expressed as mg of gallic acid equivalents (GAE)/L. The results of total anthocyanins were expressed as mg/L.

Results and discussion

Content of phenolic compounds affects wine chemical and sensory properties (colour and mouth-feel) and significantly contributes to wine quality. Since colour is one of important parameters during sensory evaluation of wine, the aim of this study was to determine the effect of thermosonication on the chromatic characteristics, but also on phenolic compounds such as anthocyanins, responsible for red colour of young wines (10). The results showed that applied treatment did not have a significant effect on the colour of wine presumably because the treatment duration was short (3 minutes only) (Table 1). However, measurements just after the treatment showed that the decrease in the content of total phenolics was higher than for total anthocyanins in all treated samples (Table 1). Change of phenolic concentration could be due to the subsequent recombination of polymers during thermosonication. This is supported by the previous research where it was shown that HPU treatment could significantly change the concentration of total phenolics (11). Variation of wine parameters such as alcohol (13 and 14 %, v/v), pH (3.5, 3.7 and 3.9) and sugar (dry wine and wine with excess sugar of 4 g/L) showed that only increase in pH from 3.5 to 3.9 caused reduction of chromatic characteristics (a, b and C) in both, treated and untreated samples (Table 1). Observed data could be due to changes in chemical forms of anthocyanins, which are strongly dependent on the pH of wine. At pH values of 3.4 to 3.6, 20-25 % of anthocyanins are in the coloured flavylium forms, but at pH values of 4, only 10 % of anthocyanins are in this form (12).

Further analysis of samples after storage of 90 days showed a change of colour as well as of phenolics and anthocyanins in treated and untreated samples (Table 2). Chromatic characteristics L, a, b and C were decreased while H value remained constant. Total phenolics and total anthocyanins content also decreased after 90 days (Table 2) compared to their content immediately after treatment. The observed changes could be due to the synergistic effect of ageing and the presence of *B. bruxellensis* yeast. The exact proportion between wine ageing and *B. bruxellensis* yeast influence is very hard to estimate. This effect is supported by other authors who also reported that phenolic compounds are unstable and participate in various types of chemical reactions during ageing (13).

Table 1. Influence of thermosonication on chromatic characteristics, total phenolics and total anthocyanins in red wine with 13 and 14 % (v/v) alcohol immediately after treatment

sample	L	a	b	C	H	TP	TA
wine with 13% (v/v) alcohol							
untreated							
pH3.5	28.56±0.12	56.32±0.10	39.19±0.13	68.61±0.16	0.61±0.00	1877.50±16.5	260.23±8.03
pH3.7	28.79±0.18	55.57±0.11	35.44±0.14	65.91±0.17	0.57±0.00	1825.33±43.58	262.3±3.34
pH3.9	28.47±0.11	51.80±0.00	29.96±0.10	59.84±0.05	0.52±0.00	1785.00±4.00	279.74±9.10
pH3.5+sugar	28.10±0.02	57.83±0.00	40.91±0.03	70.83±0.02	0.62±0.00	1869.50±18.08	273.96±2.54
pH3.7+sugar	30.01±0.05	56.89±0.00	36.99±0.06	67.86±0.04	0.58±0.00	1786.75±18.00	274.78±3.59
pH3.9+sugar	29.35±0.04	53.15±0.02	32.73±0.04	62.42±0.00	0.55±0.00	1628.00±29.00	267.62±3.64
treated							
pH3.5	28.47±0.12	57.48±0.04	38.94±0.03	69.43±0.05	0.60±0.00	1640.75±25.60	254.19±0.09
pH3.7	29.79±0.11	55.77±0.01	34.66±0.04	65.66±0.03	0.56±0.00	1552.00±32.57	248.41±0.53
pH3.9	27.59±0.12	51.36±0.04	30.06±0.13	59.51±0.10	0.53±0.00	1537.67±29.23	269.68±0.70
pH3.5+sugar	28.65±0.06	57.80±0.01	40.13±0.04	70.37±0.03	0.61±0.00	1543.50±21.18	257.95±0.53
pH3.7+sugar	28.88±0.04	55.68±0.01	36.25±0.01	66.44±0.00	0.58±0.00	1535.67±22.29	252.79±0.88
pH3.9+sugar	28.60±0.03	52.81±0.03	32.73±0.03	62.13±0.01	0.55±0.00	1535.50±8.50	265.83±2.54
wine with 14% (v/v) alcohol							
untreated							
pH3.5	27.66±0.02	57.28±0.02	39.05±0.07	72.91±0.04	0.60±0.00	1823.67±15.46	270.55±0.09
pH3.7	28.75±0.02	55.83±0.02	35.06±0.04	67.85±0.02	0.56±0.00	1744.00±22.21	281.87±0.53
pH3.9	29.13±0.01	53.08±0.02	30.78±0.07	63.71±0.02	0.53±0.00	1724.75±9.39	297.19±0.70
pH3.5+sugar	28.05±0.05	58.15±0.03	40.53±0.07	71.17±0.06	0.61±0.00	1841.75±19.50	274.36±2.54
pH3.7+sugar	28.98±0.04	56.45±0.02	36.32±0.08	67.86±0.05	0.57±0.00	1864.50±4.50	281.77±3.59
pH3.9+sugar	28.92±0.05	53.55±0.03	32.10±0.07	63.78±0.04	0.54±0.00	1716.00±12.68	288.44±3.64
treated							
pH3.5	28.04±0.01	57.49±0.01	38.98±0.01	69.46±0.00	0.60±0.00	1547.50±19.50	250.92±5.48
pH3.7	26.10±0.04	53.26±0.02	34.09±0.05	63.24±0.04	0.57±0.00	1643.00±14.00	273.18±0.81
pH3.9	30.52±0.03	53.77±0.00	30.62±0.03	61.88±0.01	0.52±0.00	1539.67±32.29	276.24±1.15
pH3.5+sugar	29.47±0.02	58.83±0.02	39.02±0.05	71.71±0.02	0.59±0.00	1615.00±9.42	269.78±0.91
pH3.7+sugar	28.55±0.04	55.47±0.01	34.88±0.04	65.52±0.03	0.56±0.00	1517.00±34.00	270.34±0.13
pH3.9+sugar	27.67±0.02	52.48±0.00	31.46±0.02	61.18±0.01	0.54±0.00	1546.33±31.69	267.34±5.47

Data presented as average value of two analytical repetitions with standard deviation. Abbreviations: L - lightness; a - redness/greenness; b - yellowness/blueness; C - chroma; H - hue angle; TP - total phenolics (mg GAE/L); TA - total anthocyanins (mg/L).

Further, *B. bruxellensis* yeast can usually convert anthocyanins into colourless pseudobase, but it can also create vinylphenolic anthocyanins (1). All mentioned processes could lead to decreased phenolics compounds and changes in wine colour. However, further study is required to confirm which process has greater influence. Variation of wine parameters such as alcohol, pH and sugar had no effect on colour or on phenolics and anthocyanins content after storage time of 90 days in all samples.

Table 2. Influence of thermosonication on chromatic characteristics, total phenolics and total anthocyanins in red wine with 13 and 14 % (v/v) alcohol after storage time of 90 days

sample	L	a	b	C	H	TP	TA
wine with 13% (v/v) alcohol							
untreated							
pH3.5	12.43±0.59	37.93±0.70	21.12±0.90	43.41±1.05	0.50±0.00	1400.66±62.47	93.28±0.26
pH3.7	19.60±0.71	45.39±1.61	33.02±2.11	56.13±2.54	0.63±0.01	1393.50±22.07	95.09±5.56
pH3.9	22.69±0.07	45.99±1.61	33.02±2.11	56.13±2.54	0.63±0.01	1301.00±47.66	98.07±5.26
pH3.5+sugar	18.12±0.31	45.93±0.39	30.25±0.49	55.00±0.60	0.58±0.00	1436.33±46.52	97.21±0.93
pH3.7+sugar	21.05±0.02	46.79±0.02	34.65±0.04	58.22±0.04	0.64±0.00	1360.75±21.04	105.53±2.26
pH3.9+sugar	18.12±0.31	45.93±0.39	30.25±0.49	55.00±0.60	0.58±0.00	1415.66±27.82	103.6±2.71
treated							
pH3.5	15.47±0.25	43.43±0.28	26.73±0.35	51.00±0.42	0.55±0.00	1598.00±11.00	106.31±3.25
pH3.7	19.72±0.41	46.64±0.51	32.99±0.67	57.12±0.80	0.62±0.00	1500.25±30.65	105.09±1.46
pH3.9	19.13±0.30	45.02±0.31	32.02±0.46	55.24±0.52	0,62±0.00	1470.67±32.19	103.80±3.40
pH3.5+sugar	13.86±1.15	40.97±1.56	23.35±1.92	47.16±2.30	0.52±0.02	1453.00±30.00	102.84±4.90
pH3.7+sugar	19.69±0.15	47.47±0.18	32.72±0.24	57.66±0.28	0.60±0.00	1441.00±3.00	116.68±2.49
pH3.9+sugar	17.85±0.50	43.27±0.65	29.62±0.80	52.44±0.99	0.60±0.01	1453.00±40.00	113.00±0.13
wine with 14% (v/v) alcohol							
untreated							
pH3.5	17.51±0.02	45.74±0.02	29.61±0.05	54.49±0.04	0.57±0.00	1446.66±14.97	85.23±0.75
pH3.7	18.75±0.13	44.88±0.12	31.41±0.20	54.78±0.22	0.61±0.00	1423.50±7.50	95.35±0.04
pH3.9	20.30±0.01	44.61±0.01	34.00±0.03	56.09±0.03	0.65±0.00	1348.66±15.06	104.13±1.23
pH3.5+sugar	16.63±0.30	44.74±0.01	28.83±1.60	53.23±0.88	0.57±0.03	1378.50±12.13	95.24±0.83
pH3.7+sugar	17.82±0.33	44.97±0.05	30.13±0.20	53.59±0.92	0.57±0.02	1350.33±33.50	93.28±2.67
pH3.9+sugar	21.39±0.24	45.50±0.12	35.30±0.35	57.59±0.31	0.66±0.00	1489.50±54.00	94.11±2.93
treated							
pH3.5	16.10±0.22	44.08±0.29	27.31±0.38	51.86±0.44	0.55±0.00	1506.70±20.54	89.25±1.67
pH3.7	17.42±0.02	44.26±0.05	29.44±0.03	53.16±0.06	0.59±0.00	1533.50±35.78	100.8±0.56
pH3.9	17.17±0.03	43.01±0.05	28.99±0.06	51.87±0.08	0.59±0.00	1427.33±15.97	107.98±3.68
pH3.5+sugar	16.40±0.09	44.65±0.11	27.74±0.15	52.56±0.18	0.55±0.00	1544.00±43.65	97.65±2.34
pH3.7+sugar	16.18±0.09	43.45±0.08	27.24±0.14	51.28±0.14	0.56±0.00	1455.50±4.50	98.73±0.52
pH3.9+sugar	15.07±0.04	40.62±0.06	25.42±0.06	47.92±0.08	0.56±0.00	1577.33±32.87	107.01±1.36

Data presented as average value of two analytical repetitions with standard deviation. Abbreviations: L - lightness; a - redness/greenness; b - yellowness/blueness; C - chroma; H - hue angle; TP - total phenolics (mg GAE/L); TA - total anthocyanins (mg/L).

Conclusions

Our study showed that thermosonication induced significant decrease in the content of total phenolics, but at the same time did not have negative effect on the colour of red wine as well as content of total anthocyanins. Further decrease in the content of total phenolics, anthocyanins and wine colour in treated and untreated samples after storage of 90 days was observed. Variation of wine parameters (alcohol, pH and sugar) in all samples (treated and untreated) had no effect on the content of phenolics and anthocyanins. Changes in pH caused change of wine colour in all samples, where influence of pH was noticeable only immediately after treatment.

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