

CONCENTRATIONS OF HIGHER ALCOHOLS DURING WINE FERMENTATION

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Abstract

The aim of the present contribution was to evaluate influence of one strain *Saccharomyces cerevisiae* on secondary aroma of Gewürztraminer wine. We focused our attention on aroma compounds, namely higher alcohols. During fermentation samples of fermentation broth were analyzed to obtain time dependent concentrations of basic analytical parameters: reduced sugars, ethanol, assimilate nitrogen, biomass. To investigate concentrations profiles of particular volatile organic compounds responsible for the sensory profile of wine, we have used two-dimensional gas chromatography by GC-TOF-MS method. Relatively simple mathematical model in form of ordinary differential equation was used for description of measured time dependent concentration profiles of higher alcohols during fermentation process. Parameters of the model for particular higher alcohols were calculated on the basis of experimental data.

Keywords: Wine aroma profile, higher alcohols, aroma compounds, alcoholic fermentation

Introduction

Wine flavor can be classified into three groups: primary (varietal), secondary (fermentative) and tertiary (wine ageing) aroma. With modern analytical methods and equipment more than 800 aroma compounds [1] such as alcohols, esters, organic acids, aldehydes, ketones, terpenes etc. have been identified in wines with a wide concentration range varying between hundreds of mg.l^{-1} to the $\mu\text{g.l}^{-1}$ levels. Their combinations form the character of wine and enable the differentiation of one wine from another.

Secondary aroma of wine originates from yeast and bacterial metabolism during alcoholic fermentation and contains various organic substances. Among the compounds influencing wine aroma, besides esters higher alcohols are very important. This contribution consequently deals with experimental measurement and mathematical modelling of higher alcohols concentration profiles during fermentation process. Redundant concentrations of higher alcohols lead to strong, pungent flavor and taste. However, its optimal concentrations give the wine fruity character [2]. At low concentrations, most of them have pleasant fruity aromas, but excessive concentrations are unwelcome and smell like organic solutions [3]. Production of higher alcohols in fermentation broth depends on many factors including species and strain of used yeast, content of amino acids in must, composition and quality of grape must, pH and temperature during fermentation, etc.

The aim of the present contribution was to find spectrum of higher alcohols produced by one commercial *Saccharomyces cerevisiae* strain designated as *Oenoferm Belle Arome* (produced by company Erbslöh Geisenheim, Germany). This strain of yeast was used as starter for fermentation process. As fermentative medium real grape must variety Gewürztraminer was used. Total concentration of reduced sugars in the must was 225 g.l^{-1} . Fermentation temperature was 17°C and above mentioned strain of *S. cerevisiae* produced individual metabolites in different concentrations and combinations which significantly influenced resulting wine flavor. To investigate time concentrations profiles of particular higher alcohols we have used liquid-liquid extraction of samples followed by GC-TOF-MS method [3].

Inside the fermentation broth we identified completely 19 various higher alcohols. Relatively simple mathematical model in comparison with literature (for example [4]) was derived. Model has the form of ordinary differential equation and was used for description of measured time dependent profiles of each higher alcohol during fermentation process. Parameters of the model for particular higher alcohols were calculated on the basis of experimental data.

1. MATERIALS AND METHODS

1.1. Materials



In experiment, one yeast strain of *Saccharomyces cerevisiae* was used. *S. cerevisiae* Oenoferm belle arôme is a commercial preparation of dry wine yeast produced by company Erbslöh Geisenheim, Germany. This strain is characterized by high production of aromatic compounds and is suitable for production of white wines with expressive fruity aroma.

For the experimental fermentation grapes of vine variety Gewürztraminer was used. Destemmed and crushed grapes were macerated for 4 hours and subsequently pressed. Clarification of pressed grape juice was performed statically using bentonite and must gelatine (preparation Mostgelatine, Erbslöh Geisenheim, Germany) (dose 1 g.l⁻¹). After 12 h clarified juice was treated by gaseous SO₂ (10 mg.l⁻¹), filled into 35 l glass flasks and inoculated by yeast strain Oenoferm belle arôme in form of liquid yeast starter. Starting concentration of biomass in grape juice was 10⁶ cells/ml. The main alcoholic fermentation had proceeded for 2 weeks at the temperature 17°C. Layout of experimental fermentation is depicted in Fig.1.

Fig. 1: Experimental layout

1.2. Methods

At regular time intervals (every 24 h), samples of fermenting medium were taken and analyzed. Before sampling, content of fermenter was shortly stirred to homogenize the yeast biomass. After determination of biomass concentration samples were centrifuged and analyzed in terms of the other analytical parameters.

Basic oenological parameters as well as detailed higher alcohols profile have been determined. Concentrations of reducing sugars have been analyzed by Schoorl method, concentration of ethanol and extract pycnometrically (OIV-MA-AS312-01A, OIV-MA-AS2-03B). Profiles higher alcohols were analyzed by comprehensive two-dimensional gas chromatography using method presented in [5]. By the analysis, benzophenone was used as the internal standard (20 µL of methanol solution of benzophenone with concentration 16 mg.l⁻¹ was added to 6 ml of sample). For bioengineering modeling relative concentrations c_{rel} in µg.l⁻¹ of individual VOCs were used.

2. MATHEMATICAL MODEL

Mathematical model was derived on the basis of mass balance of higher alcohol i in fermentation broth during very small time interval dt , which verbal designation is as follows

$$\left\{ \begin{array}{c} \text{accumulation} \\ \text{of higher} \\ \text{alcohol } i \\ \text{in system} \\ \text{during time } dt \end{array} \right\} = \left\{ \begin{array}{c} \text{source of} \\ \text{higher} \\ \text{alcohol } i \\ \text{in system} \\ \text{during time } dt \end{array} \right\} - \left\{ \begin{array}{c} \text{output of} \\ \text{higher} \\ \text{alcohol } i \\ \text{from system} \\ \text{during time } dt \end{array} \right\} \quad (1)$$

Mathematical formulation of Eq. (1) is possible to write as

$$\frac{dc_i^l}{dt} = k_{1,i}c_x - k_{2,i}c_i^l \frac{\dot{m}_{CO_2}}{V_{must}} \quad (2)$$

Significance of symbols in Eq.(2) is:

c_i^l - concentration of higher alcohol i in the liquid phase of fermentation broth

c_x - concentration of biomass

$\dot{m}_{CO_2}$ - mass flow rate of carbon dioxide

V_{must} - volume of the fermentation broth

$k_{1,i}$ and $k_{2,i}$ - kinetic constants.

Kinetic constant $k_{1,i}$ is proportionality constant of higher alcohol formation and therefore is proportional to biomass concentration inside the fermentation broth. Kinetic constant $k_{2,i}$ is proportional to downfall of higher alcohol amount in fermentation broth, mainly caused by its elutriation from liquid phase with carbon dioxide. But descending concentration of particular higher alcohol may be caused not only by desorption with carbon dioxide. Inside the fermentation broth are present organic acids too and higher alcohols can react with them and produce esters, because acid environment enables esterification. Both two above mentioned processes are possible respond for decreasing of higher alcohol concentration in liquid phase and next observation is needed on this field.

Ordinary differential equation (2) was solved by numerical method of Runge Kutta 4-th level with known initial condition of concentration of higher alcohol in the grape must. Magnitude of velocity constants $k_{1,i}$ and $k_{2,i}$ during calculations were changed with target to obtain minimum sum of squares between experimental and calculated values of higher alcohol concentration profile.

3. RESULTS

Generally speaking, results of experimentally measured concentration profiles display two basic behaviour. In first group of higher alcohols concentration increases with time and in second group concentration decreases with time during fermentation process. Numerical values of experimental profiles each identified higher alcohols are presented in following two tables.

Higher alcohol	Time [h]							
	0	24	48	96	144	192	240	288
Isoamyl alcohol	0,00	25,00	163,24	378,92	429,33	598,14	521,51	514,98
1-dodecanol	1,51	1,60	2,89	5,93	2,83	4,81	4,00	5,43
1-hexadecanol	0,00	0,00	0,66	1,17	1,95	2,43	2,19	2,25

2,3-butanediol	0,00	0,00	0,00	15,43	20,41	63,09	68,58	94,46
2-phenylethyletanol	136,65	128,00	111,06	133,43	121,82	166,12	140,72	142,28

Table 1. Concentration of higher alcohols with increasing profiles [$\mu\text{g l}^{-1}$], time in hours

Higher alcohol	Time [h]							
	0	24	48	96	144	192	240	288
1-hexanol	216,19	175,00	127,99	40,11	23,05	31,13	20,22	19,65
1-nonanol	2,94	2,80	2,49	0,72	0,22	0,00	0,00	0,00
1-octanol	13,82	13,70	13,70	12,46	11,00	9,63	6,80	7,18
1-propanol-3-metyltio-	1,54	1,00	0,83	0,16	0,27	0,46	0,27	0,24
2-nonanol	11,24	6,00	3,64	0,00	0,00	0,00	0,00	0,00
6-metyl-5-hepten-2-ol	8,40	4,50	2,62	0,45	0,00	0,29	0,28	0,00
Benzylalcohol	2,37	2,40	2,57	1,76	1,19	1,40	1,12	1,36
1-heptanol	43,44	25,00	10,34	1,74	0,73	1,21	0,73	0,76
1-octen-3-ol	25,47	18,00	8,00	1,61	0,00	0,00	0,00	0,00
2-octanol	9,44	5,00	3,40	0,00	0,00	0,00	0,00	0,00
2-octen-1-ol	2,53	1,00	0,00	0,00	0,00	0,00	0,00	0,00
3-hexen-1-ol	0,74	0,50	0,00	0,00	0,00	0,00	0,00	0,00
3-octanol	9,53	6,50	2,79	0,00	0,00	0,00	0,00	0,00
2-ethyl-1-hexanol	6,85	7,30	7,89	1,68	0,00	0,86	0,80	1,74

Table 2. Concentration of higher alcohols with decreasing profiles [$\mu\text{g l}^{-1}$], time in hours

As it mentioned above, on the basis of measured concentration profiles we made evaluation of kinetic parameters using mathematical model (2). Numerical results of calculations are shown in following Table 3.

Higher alcohol	$k_{1,i} 10^7$ [h ⁻¹]	$k_{2,i} \cdot 10^7$ [m ³ kg ⁻¹ h ⁻¹]	Correlation coefficient
Isoamyl alcohol	120,6777	6,9496	0,7938
1-dodecanol	0,5286	7,2524	0,8323
1-hexadecanol	10,3674	4,1676	0,9903
2,3-butanediol	0,0689	0,0089	0,6273
2-phenylethyletanol	0,3947	0,0128	0,4164

Table 3. Kinetic constants $k_{1,i}$ and $k_{2,i}$ and correlation coefficients for alcohols with increasing profiles

On the figures below one can see how the experimental values of higher alcohol concentration changing with time during fermentation (red points) and result of mathematical modelling (solid line). For illustration we can see graphs of 2,3-butanediol and isoamylalcohol on Fig. 1.

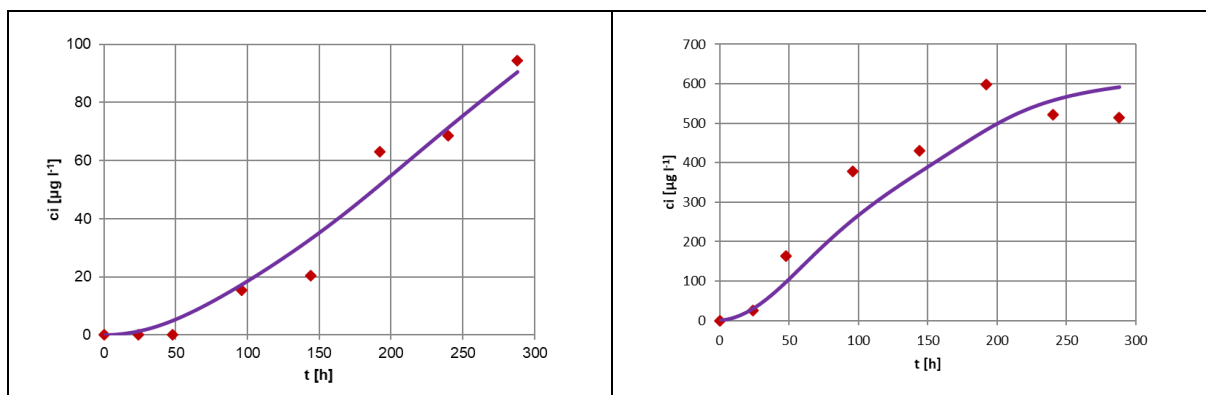


Fig. 1. Experimental and calculated concentration profile: 2,3-butanediol (left), isoamylalcohol (right)

Next Table 4 lists the results of calculation for higher alcohols with decreasing concentration profiles, kinetic constants $k_{1,i}$ and $k_{2,i}$.

Higher alcohol	$k_{1,i} \cdot 10^9$ [h ⁻¹]	$k_{2,i} \cdot 10^9$ [m ³ kg ⁻¹ h ⁻¹]	Correlation coefficient
1-hexanol	66,2339	53,4517	0,9961
1-nonanol	0	38,8690	0,9933
1-octanol	0	6,5661	0,9087
1-propanol-3-metyltio-	1,4046	83,7687	0,9596
2-nonanol	0,0742	188,2402	0,9924
6-metyl-5-hepten-2-ol	1,2043	159,2696	0,9919
Benzylalcohol	0,1886	9,6154	0,9640
1-heptanol	6,5613	171,0511	0,9987
1-octen-3-ol	0,1993	122,3713	0,9987
2-octanol	0,0952	146,3906	0,9891
2-octen-1-ol	0	329,8165	0,9985
3-hexen-1-ol	0	206,6681	0,9826
3-octanol	0	133,2050	0,9999
2-ethyl-1-hexanol	0,9311	30,1755	0,9519

Table 4. Kinetic constants $k_{1,i}$ and $k_{2,i}$ and correlation coefficients for alcohols with decreasing profiles

On Fig. 2 we have depicted experimental (red points) and calculated (solid line) concentration profiles of higher alcohol changing with time during fermentation. For illustration we introduce two compounds only: of 1-heptanol and 1-hexanol.

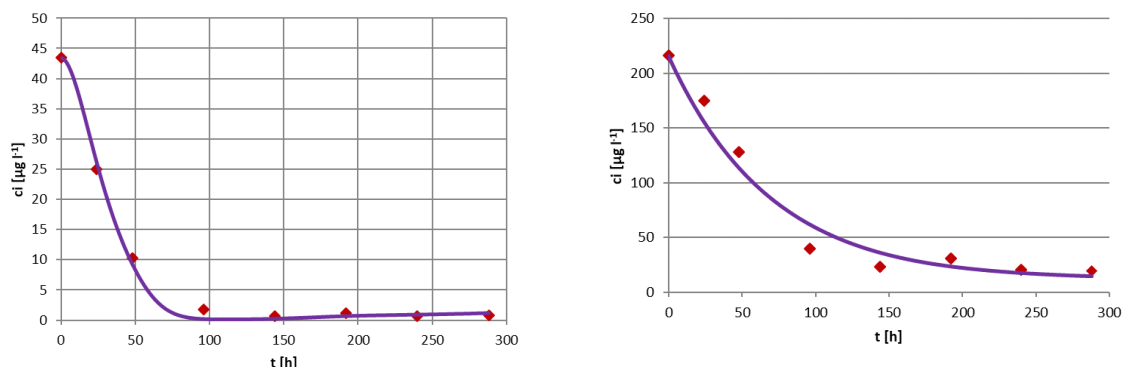


Fig. 2. Experimental and calculated concentration profile: 1-heptanol (left), 1-hexanol (right)

4. DISCUSSION

On the basis of experimental measurements and mathematical modelling results noted above we can say, that simple mathematical model Eq.(2) has ability amazingly good describe time evolution of aromas. For higher alcohols with decreasing concentrations during wine fermentation the agreement between experimental and calculated values is very good – see values of correlation coefficients listed in table 4, where its values are in the range 0,95 – 0,99. In the case of higher alcohols with ascending concentrations during fermentation the coincidence between experimental and calculated values is a little bit worse but always satisfactory. Corresponding values of correlation coefficients are in the range 0,41 – 0,99.

On this place it is necessary to remark, that numerical values of kinetic constants $k_{1,i}$ and $k_{2,i}$ in mathematical model are very small. The reason is, that values of aromas concentrations are in $\mu\text{g l}^{-1}$.

As it was mentioned above, we investigated two groups of time evolution of aromas. Higher alcohols with ascending concentrations was observed in five cases only – see table 1. The most important compounds from this group are Isoamyl alcohol and 2,3-butanediol. This two compounds have maximal final concentration values. The only reason for ascending time values of aromas in this group is activity of yeasts. Production of the species listed in table 1 has to be very high, because in spite of elutriation this compounds by carbon dioxide its concentration in liquid phase rising.

Group of compounds with time descending concentration during fermentation process is more numerous with 14 various higher alcohols – see table 2. The largest final concentrations have 1-hexanol and 1-octanol. All other compounds detected from chromatograms have so small final concentration in liquid phase, that are due to higher perception threshold not sensory registered. For the reason of time descending concentration are few possibilities. In the first case it is elutriation of aromas by carbon dioxide from liquid phase. Next reason may be chemical reaction of higher alcohols present in liquid phase with organic acids to form esters. Up to now we have no confirmation if esterification occurs or not and if yes what is its magnitude. For approve this it is necessary make next special experiments.

5. CONCLUSIONS

In present article we are concerned with experimental measurement and mathematical modelling of time evolution of higher alcohols during real wine must alcoholic fermentation. To investigate concentrations profiles of higher alcohols particularly responsible for the sensory profile of wine, we have used two dimensional gas chromatography by GC-TOF-MS method. This method enable to detect very low concentrations of aroma compounds in $\mu\text{g l}^{-1}$. Inside fermentation broth we detected total 19 various higher alcohols, 5 of them with increasing time profile and 14 with decreasing time profiles of aromas. Kinetic parameters to proposed mathematical model was evaluated and good agreement between experimental and calculated data was obtained.

ACKNOWLEDGMENTS

This work was supported by the Slovak Research and Development Agency under the contract APVV-17-0109.

REFERENCES

1. Furdíková, K Makyšová, K Ďurčanská, K Špánik, I Malík, F 2014 “Influence of yeast strain on aromatic profile of Gewürztraminer wine”, *LWT – Food Science and Technology* 59, pp. 256-262.
2. Swieger, JH Pretorius, IS 2005, “Yeast modulation of wine flavor”, *Advances in Applied Microbiology*, 57, pp. 131-175.
3. Furdíková, K Bajnociová, L Malík, F Špánik, I 2017, “Investigation of volatile profile of varietal Gewürztraminer wines using two-dimensional gas chromatography”, *Journal of Food and Nutrition Research*, 56 (1), pp. 73-85.
4. Bartsch, J Borzi, A Schenk, Ch Schmidt, D Müller, J Schulz, V Velten, K 2019, “An extended model of wine fermentation including aromas and acids”, *eprint arXiv:1901.03659*, pp. 2-11.