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ГОДИНА 12 **VOLUME XII**

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FACULTY OF AGRICULTURE

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ПРЕДГОВОР

Публикувањето на дванаесеттото издание на Годишниот зборник на Земјоделски факултет при Универзитет „Гоце Делчев" – Штип, 2014, вол. 12, е уште еден евидентен доказ за посветеноста на нашиот факултет во науката и нејзината апликација во земјоделството.

Дванаесеттото издание на Годишниот зборник на Земјоделски факултет е прво издание кое во целост е изведувано преку електронскиот систем УГД журнали достапен на веб-страницата на УГД, на линкот

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Современите информатички и комуникациски технологии, како и новите техники за научно истражување, наложија промовирање на електронски пристап во публикувањето на резултатите од научноистражувачката дејност на Универзитетот. Тоа создаде потреба да се користи нов и современ пристап во издаваштвото со употреба на моќни алатки како што се е-журнали и е-библиотека на УГД.

Науката е примарен фактор за конструктивен развојот на секоја област од современото општество. Научниот кадар од Земјоделскиот факултет постојано ги следи новите достигнувања во науката и современото земјоделие и ги имплементира новите трендови во научно-стручните истражувања како и во студиските програми од сите три циклуси. Од сето тоа произлегуваат дванаесетте изданија на Годишен зборник, акредитирани повеќе студиски програми за сите циклуси на студирање на Земјоделскиот факултет, бројни проекти домашни и меѓународни, учество на престижни научни и стручни манифестации на научниот кадар од факултетот, и бројни достигнувања и успешна примена на науката во соодветната земјоделска практика.

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ОПРЕДЕЛУВАЊЕ НА SO_2 И РЕДУЦИРАЧКИ ШЕЌЕРИ ВО МАКЕДОНСКИ ВИНА

Виолета Иванова-Петропулос[1]*, Саша Митрев[1]

Краток извадок

SO_2 делува како антиоксидант, спречувајќи ја активноста на оксидазите, но и како антимикробен агенс, а покажува и потенцијал за обелување на пигментите и елиминација на непријатни мириси кај вината. Глукозата и фруктозата се главните јаглехидрати во грозје и вино, кои вообичано се нарекуват редуцирачки шеќери. Во ова истражување е извршена проверка на титрациски методи за определување на SO_2 (слободен и вкупен) и на редуцирачки шеќери во вина. Линеарноста, точноста и прецизноста на методите беше потврдена со примена на стандардни раствори од SO_2 и редуцирачки шеќери (фруктоза и гклукоза) подготвени во определен концентрациски опсег, како и со ниски, средни и високи концентрации. Дополнително, точноста на методите е потвдена со методата на стандардни додатоци. Беа проверени и повторливоста и репродуцибилноста на методите со титрација на реални примероци вина, анализирани со соодветен број на повторувања. Валидираните методи се применети за определување на содржината на слободен и вкупен SO_2, како и редуцирачки шеќери во десет вина од сортата *вранец*, произведени со различни квасци за ферментација.

Клучни зборови: *SO_2, редуцирачки шеќери, вино, титрација*

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DETERMINATION OF SO_2 AND REDUCING SUGARS IN MACEDONIAN WINES

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Apstract

SO_2 acts as an effective antoxidant, preventing the activity of the oxidases and antimicrobial agent, showing a potential for bleaching the pigments and elimination of unpleasant odour in wines. Glucose and fructose are the main carbohydrates in grapes and wine, usually called reducing sugars. In this study, three titrimetric methods for determination of SO_2 (free and total) and reducing sugars in wines were checked. The linearity, accuracy and precision of the methods were confirmed using standard solution of SO_2 and reducing sugars (fructose and glucose) prepared in appropriate concentration range, as well as with low, medium and high concentrations. Additionally, the accuracy of the methods was confirmed by standard additions. Repeatability and reproducibility were confirmed with titration of real samples, analyzed with appropriate repetitions. Validated methods were applied for determination of the content of free and total SO_2, as well as reducing sugars in ten Vranec wines produced with different yeasts for fermentation.

Keywords: *SO_2, reducing sugars, wine, titration*

1. Introduction

Wine is a complex mixture of a large number of compounds including carbohydrates, alcohols, aldehydes, esters, acids, proteins and vitamins. It also contains a number of elements, polyhydroxy aromatic and polyphenolic compounds, such as tannins, anthocyanins and flavonols, which contribute highly to colour and taste [1-5].

Sulfur dioxide is naturally present in wine, which can be produced at concentrations up to 64 mg/L by the yeast metabolism [6]. However, most of the yeasts cannot produce more than 10 mg/L SO_2, so that contents of SO_2 higher than 30 mg/L usually are result of doses added during the vinification. The use of SO_2 in winemaking is due to its ability of an effective antoxidant, preventing the activity of the oxidases. Also, it has significant activity as antimicrobial agent, as well as potential for bleaching the pigments and elimination of unpleasant odours (as a result of oxidation). Because yeasts are very sensitive to SO_2 (also, to other stress factors), it can selectively act against the wild yeasts, which come from the grape skin or equipment in the winery, and stop their activity. Sulfur dioxide can be added in a form of a salt, potassium metabisulphate ($K_2S_2O_5$), which can be ionized in acid media, releasing gaseous SO_2.

Sulfur dioxide is present in wines as free and total SO_2. SO_2 that is bound to aldehydes (acetaldehyde), sugars, tannins and anthocyanins is bound SO_2. Free and bound SO_2 in wine exist in a dynamic equilibrium:

$$SO_2 \text{ (free)} \longleftrightarrow SO_2 \text{ (bound)}$$

Only free SO_2 possesses antiseptic and antioxidant properties. Higher amounts of SO_2 negatively influence the wine quality (flavor and taste).

The content of SO_2 (free and total) is usually determined by iodine titration, according to the Ripper's method [7], using standard solution of iodine in presence of stretch as an indicator and sulfuric acid. Before titration, solution of NaOH is used in order to release the bound SO_2. Iodine reacts with sulfur dioxide in the following way:

$$SO_2 + I_2 + 2H_2O \longrightarrow H_2SO_4 + 2HI$$

In addition, glucose and fructose are the main carbohydrates in grapes and wine, usually called reducing sugars. The content of sugars in grapes depends on variety, maturity and health conditions. Varieties of *Vitis vinifera* accumulate about 20 % sugars and even more during the ripening phase, while varieties from *Vitis labrusca* and *Vitis rotundifolia* rarely achieve this level of sugars.

During fermentation, sugars are broken down by the action of the yeast, thus forming an alcohol (ethanol) and carbon dioxide:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

The ratio of glucose/fructose decreases from 0.95 initially to 0.25 at the end of fermentation. In fact, the glucose ferments at the beginning since it is used by different yeasts, which means that fructose is more prevalent then glucose. Dry wines contain residual sugar whose content is less than 1.5 g/L. At this concentration, which is low, the sweetness of wine is not felt.

For determination of reducing sugars in must and wine, chemical methods based on reduction-oxidation (redox) reactions that take place between sugars and Fehling's solution, are usually applied. Fehling's solution contains copper (II) ions that can be reduced by some sugars to copper (I) ions. This reaction can be used for the quantitative analysis of reducing sugars.

The aim of this work is validation of the methods for determination of SO_2 and reducing sugars in wines using titration methods, and then, application of the methods for analysis of different wine samples from Macedonia.

2. Materials and methods

2.1. Reagents

Standard solution of SO_2 and standards of glucose and fructose were purchased from Sigma Aldrich (St. Louis, MO). All other reagents used were with analytical grade of purity.

2.2. Wine samples

In total, 10 red wines from Vranec variety (vintage 2013) produced with different yeasts for fermentation were analyzed.

2.3. Determination of SO_2

Free SO_2. A volume of 50 mL wine is transferred to flask of 250 mL, followed by addition of 10 mL 25 % (*v*/*v*) solution of sulfuric acid (1+3) and 2-3 mL 1 % solution of stretch as an indicator. Sulfuric acid is added since the oxidation in acid conditions is more intensive. The prepared wine is titrated with a standard solution of iodine with concentration of 0.01 mol/L until the endpoint of titration (change of color to dark-blue). The following equation is used for calculation of the content of free SO_2:

$$\text{Free } SO_2/\text{mg/L} = V(I_2) \cdot c(I_2) \cdot M(SO_2) \cdot 1000/V(\text{wine})$$
$$\text{Free } SO_2/\text{mg/L} = V(I_2) \cdot 12.8$$

Total SO_2. A volume of 25 mL of 1 M NaOH is transferred to flask of 250 mL, followed by addition of 50 mL wine. The sample is mixed, closed with a rubber stopper and left for 10 min in a dark place. Then, 10 mL 25 % (*v*/*v*) solution of sulfuric acid (1+3) and 2-3 mL 1 % solution of stretch are added and the sample is titrated with standard solution of iodine (0.01 mol/L) until the endpoint of titration (change of color to dark-blue). The following equation is used for calculation of the content of total SO_2:

$$\text{Total } SO_2/\text{mg/L} = V(I_2) \cdot c(I_2) \cdot M(SO_2) \cdot 1000/V(\text{wine})$$
$$\text{Total } SO_2/\text{mg/L} = V(I_2) \cdot 12.8$$

The content of SO_2 (free or total) can be directly read out from Table 1, using the consumed volume of I_2 for titration of the sample.

2.4. Determination of reducing sugars

For determination of reducing sugars, wine is diluted 10 times and then, 10 mL of the diluted wine is transferred to a flask (250 mL) that contains 10 mL Fehling I and 10 mL Fehling II solutions. The flask with the sample is heated on a moderate temperature until boiling temperature (or until appearance of 1-2 bubbles), followed with a change of color to red-brown (depending on the sugar content in wine). After heating, the flask is cooling (under tap water), and then, 10 mL 20 % (*m/v*) solution of KI and 10 mL 25 % (v/v) sulfuric acid are added to the flask. The flask is closing with a rubber stopper and left in a dark place to stand 2-3 min. Then, 2-3 mL of 1 % solution of stretch is added and the sample is titrated with 0.1 mol/L solution of $Na_2S_2O_3$ until change of color from yellow-brown to milky-white. Previously, a blank sample should be prepared and titrated in a same way as wine, using distilled water (20 mL). Then, the total consumed volume of $Na_2S_2O_3$ is calculated as a difference between the volumes of $Na_2S_2O_3$ consumed for titration of the blank and wine:

$$V(Na_2S_2O_3) = V(Na_2S_2O_3)_{\text{слепа проба}} - V(Na_2S_2O_3)_{\text{вино}})$$

and used for determination of the value for sugars content, found in a table (Table 2).

Table 1. Table for SO_2 (mg/L) in wine

$V(I_2)$/ mL	0	1	2	3	4	5	6	7	8	9
0	0.00	1.28	2.26	3.84	5.12	6.40	7.68	8.96	10.24	11.52
1	12.80	14.08	15.36	16.64	17.92	19.20	20.48	21.76	23.04	24.32
2	25.60	26.88	28.16	29.44	30.72	32.00	33.28	34.56	35.84	37.12
3	38.40	39.68	40.96	42.24	43.52	44.80	46.08	47.36	48.64	49.92
4	51.20	52.48	53.76	55.04	56.32	57.60	58.88	60.16	61.44	62.72
5	64.00	65.28	66.56	67.84	69.12	70.40	71.68	72.96	74.24	75.52
6	76.80	78.08	79.36	80.64	81.92	83.20	84.48	85.76	87.04	88.32
7	89.60	90.88	92.16	93.44	94.72	96.00	97.28	98.56	99.84	101.12
8	102.40	103.68	104.96	106.24	107.52	108.80	110.08	111.36	112.64	113.92
9	115.20	116.48	117.76	119.04	120.32	121.60	122.88	124.16	125.44	126.72
10	128.00	129.28	130.56	131.84	133.12	134.40	135.68	136.96	138.24	139.52
11	140.80	142.08	143.36	144.64	145.92	147.20	148.48	149.76	151.04	152.32
12	153.60	154.88	156.16	157.44	158.72	160.00	161.28	162.56	163.84	165.12
13	166.40	167.68	168.96	170.24	171.52	172.80	174.08	175.36	176.64	177.92
14	179.20	180.48	181.76	183.04	184.32	185.60	186.88	188.16	189.44	190.72
15	192.00	193.28	194.56	195.84	197.21	198.40	199.68	200.96	202.24	203.52
16	204.80	206.08	207.36	208.64	209.92	211.20	212.48	213.76	215.04	216.32
17	217.60	218.88	220.16	221.74	222.72	224.00	225.28	226.56	227.84	229.12
18	230.40	231.68	232.96	234.24	235.52	236.80	237.08	238.36	239.64	240.92
19	243.20	244.48	245.76	247.04	248.32	249.60	250.88	252.16	253.44	254.72
20	256.00	257.28	258.56	259.84	261.12	262.40	263.68	264.96	266.24	267.52
21	268.80	270.08	271.36	272.64	273.92	257.20	276.48	277.76	279.04	280.32

Table 2. Table for reducing sugars (g/L) in wine

$V(Na_2S_2O_3)$/ mL	0	1	2	3	4	5	6	7	8	9
0	0.0	0.3	0.6	1.0	1.3	1.6	1.9	2.2	2.6	2.9
1	3.2	3.5	3.8	4.2	4.5	4.8	5.1	5.4	5.7	6.1
2	6.4	6.7	7.1	7.4	7.7	8.1	8.4	8.7	9.0	9.4
3	9.7	10.0	10.4	10.7	11.0	11.4	11.7	12.0	12.3	12.7
4	13.0	13.3	13.7	14.0	14.4	14.7	15.0	15.4	15.7	16.1
5	16.4	16.7	17.1	17.4	17.8	18.1	18.4	18.8	19.1	19.5
6	19.8	20.1	20.5	20.8	21.2	21.5	21.8	22.2	22.5	22.9
7	23.2	23.5	23.9	24.2	24.6	24.9	25.2	25.6	25.9	26.3
8	26.5	26.9	27.3	27.6	28.0	28.3	28.6	29.0	29.3	29.7
9	29.9	30.3	30.7	31.0	31.3	31.7	32.0	32.7	32.7	33.0
10	33.4	33.7	34.1	34.4	34.8	35.1	35.4	35.8	36.1	36.5
11	36.8	37.2	37.5	37.9	38.2	38.6	38.9	39.3	39.6	40.0
12	40.3	40.7	41.0	41.4	41.7	42.1	42.2	42.8	43.1	43.5
13	43.8	44.2	44.5	44.9	45.2	45.6	45.9	46.3	46.6	47.0
14	47.3	47.7	48.0	48.4	48.7	49.1	49.4	49.8	50.1	50.5
15	50.8	51.2	51.5	51.9	52.2	52.6	52.9	53.3	53.6	54.0
16	54.3	54.7	55.0	55.4	55.8	56.2	56.5	56.8	57.3	57.6
17	58.0	58.4	58.8	59.1	59.5	59.9	60.3	60.7	61.0	61.4
18	61.8	62.2	62.5	62.9	63.3	63.7	64.0	64.4	64.8	65.1
19	65.5	65.9	66.3	66.7	67.1	67.5	67.8	68.2	68.6	69.1
20	69.4	69.8	70.2	70.6	71.0	71.4	71.7	72.1	72.5	72.9
21	73.3	73.7	74.1	74.5	74.9	75.3	75.6	76.0	76.4	76.8
22	77.2	77.6	78.0	78.4	78.8	79.2	79.6	80.0	80.4	80.8
23	81.2	81.6	82.0	82.4	82.8	83.2	83.6	84.0	84.4	84.8
24	85.2	85.6	86.0	86.4	86.8	87.2	87.6	88.0	88.4	88.8
25	89.2	89.6	90.0	90.4	90.8	91.2	91.6	92.0	92.4	92.8

3. Results and discussion

3.1. Methods validation

Linearity, accuracy, precision, repeatability and reproducibility were checked for SO_2 and reducing sugars in wine, considering the complete analytical procedures. For quantitative analysis of SO_2 and reducing sugars, we used standard solutions of SO_2 and carbohydrates (glucose and fructose), respectively.

Linearity. The linearity data of the analytical methods for determination of free SO_2, total SO_2 and reducing sugars are presented in Table 3. Each concentration level was analyzed in triplicate. Linearity was satisfactory in all cases with correlation coefficients (R^2) of 0.9999.

Table 3. Intercept, slope and correlation coefficients (R^2)

Compound	Intercept	Slope	R^2	Range
SO_2^*	0.4307	0.9943	0.9999	0-500 (mg/L)
SO_2^{**}	0.3512	0.9957	0.9999	0-500 (mg/L)
Reducing sugars	0.0603	0.9987	0. 9999	0-100 (g/L)

*SO_2 determined with the procedure for free SO_2
*SO_2 determined with the procedure for total SO_2
Reducing sugars: glucose+fructose

Accuracy and precision. The intra-day and inter-day accuracy and precision were determined with titration of standard solutions of SO_2 and reducing sugars with low, medium and high concentration (Table 4). For determination of intra-day accuracy and precision, freshly prepared solutions were used, analyzed immediately, in 10 repetitions during the day. Inter-day accuracy and precision were determined with titration of the standard solutions during 10 consecutive days. The accuracy was determined with calculation of the relative error of the determined concentration compared with the true (nominal) value. Precision was expressed as relative standard deviation (*RSD*). Results for inter-day and intra-day accuracy and precision are presented in Table 4. Relative errors are ranged between -0.16 to 0.86 %, and relative standard deviations in range of 0.47 to 5.95 %. Obtained results confirm that the suggested methods for determination of SO_2 (free and total) and reducing sugars are accurate and convenient for quantitative analysis.

Table 4. Intra- and Inter- day accuracy and precision data for standard solutions of SO_2 and reducing sugars (n=10)

Sample	SO_2						Reducing sugars					
	10 mg/L		50 mg/L		100 mg/L		10 mg/L		50 mg/L		100 mg/L	
	Found	e_R(%)	Found	e_R(%)	Found	e_R(%)	Found	e_R (%)	Found	e_R (%)	Found	e_R(%)
Intra- day accuracy and precision												
<*x*>	9.98	-0.16	50.17	0.35	100.22	0.22	10.15	1.5	50.23	0.46	50.23	0.04
SD	0.51		0.51		0.58		0.32		0.24		0.24	
***RSD* (%)**	5.13		1.02		0.58		3.12		0.47		0.47	
Inter- day accuracy and precision												
<*x*>	9.86	-1.44	50.67	1.37	100.74	0.73	10.04	0.4	10.04	0.06	10.04	0.86
SD	0.58		0.63		0.59		0.31		0.31		0.31	
***RSD* (%)**	5.95		1.24		0.58		3.12		3.12		3.12	

Labels: <*x*> - average, SD – standard deviation, RSD – relative standard deviation

The accuracy of the methods was checked using the standard addition method. One red and one white wine sample, previously analyzed, were spiked with appropriate volumes of the standard solutions of SO_2 and reducing sugars (glucose and fructose) with concentration of 5, 10 and 50 mg/L for each standard. The satisfactory results for the recovery ranging from 92.5–105% (Table 5) confirmed that the methods are accurate and convenient for quantitative analysis.

Table 5. Results from the standard additions method for checking the accuracy of the titration methods for determination of SO_2 (free and total) and reducing sugars in wine samples (n = 3)

	γ (Free SO_2)			*γ* (Total SO_2)			*γ* (Reducing sugars)		
White wine Standard addition	Calcu lated /mg/L	Found /mg/L	Reco very, %	Calcu lated /mg/L	Found /mg/L	Reco very, %	Calcu lated /mg/L	Found /mg/L	Recovery, %
I	33.16	32.00	96.50	88.20	89.60	101.6	11.40	11.53	101.1
II	38.16	37.12	97.27	93.20	92.16	98.88	16.40	15.36	93.66
III	78.16	76.80	98.26	133.20	131.8	98.98	56.40	55.04	97.59
Red wine									
I	19.08	19.20	100.6	58.76	60.16	102.4	8.84	8.96	101.4
II	24.08	24.32	100.9	63.76	66.56	104.4	13.84	12.80	92.49
III	64.08	64.00	99.8	103.76	104.9	101.3	53.84	55.04	102.2

Repeatability and reproducibility. Additionally, to confirm the accuracy of the methods and to check their repeatability, 10 repetitions in one day were performed on two real samples (one red and one white wine). Results are presented in Table 6. As can be seen from the table, the values for the standard deviations for repeatability for all three methods are very low (SD = 0.14 to 0.66 for white wine and SD = 0.17 to 0.54 for red wine), which confirms that they are accurate and can be applied for determination of SO_2 (free and total) and reducing sugars in white and red wines.

Table 6. Results for repeatability and reproducibility of SO_2 (free and total) and reducing sugars in white (Smederevka) and red (Vranec) wines

Sample	**Content of free SO_2/mg/L**	**Content of total SO_2/mg/L**	**Content of reducing sugars/g/L**
Smederevka	**Repeatability** (10 replicates in one day)		
<*x*>	28.93	82.56	6.49
SD	0.66	0.67	0.14
RSD (%)	2.28	0.82	2.23
Vranec			
<*x*>	13.82	53.50	3.72
SD	0.54	0.54	0.17
RSD (%)	3.90	1.01	4.53
Smederevka	**Reproducibility** (3 replicates x 3 titrations x 5 days)		
<*x*>	29.01	82.77	6.60
SD	0.74	0.74	0.17
RSD (%)	2.55	0.89	2.62
Vranec			
<*x*>	13.65	53.33	3.67
SD	0.74	0.74	0.23
RSD (%)	5.41	1.39	6.30

<*x*> - average, SD – standard deviation, RSD – relative standard deviation

Reproducibility was also checked with replicate samples analyzed in five different days (3 replicates x 3 titrations x 5 days) and the *RSD* for each parameter was calculated (Table 6). Accordingly, the method showed good repeatability and reproducibility and the values for *RSD* were <10% (most of the values were <5 %).

3.2. Application of methods for analysis of Vranec wines fermented with different yeast

Ten Vranec wines were fermented with different *Saccharomyces cerevisiae* yeast strains: Clos, RC212, D254 and BDX (from Lallemand), and Vinalco (from Bitola, R. Macedonia) and analyzed for determination of SO_2 and reducing sugars. Results are presented in Table 7. It was noticed that the content of free and total SO_2 ranged in quantities sufficient to protect the wines from oxidation and other microbial infections. Thus, the amount of free SO_2 ranged from 24.32 to 26.88 mg/L, the total SO_2 ranged from 51.2 to 67.84 mg/L, while the content of reducing sugars was not very different between the wines (ranged from 5.4 to 6.1 g/L), which means that the rate of alcoholic fermentation was similar in all wines where different yeast cells were used to ferment glucose into ethanol.

Table 7. Content of SO_2 (free and total) and reducing sugars in Vranec wines fermented with different yeasts for fermentation

Wines	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Reducing sugars (g/L)
V-L1	26.88	53.76	2.9
V-L2	23.04	57.60	2.6
V-L3	24.32	62.72	3.2
V-L4	25.60	52.48	3.2
V-Vi1	32.00	51.2	2.6
V-Vi2	24.32	58.88	2.6
V-Vi3	23.04	55.04	2.0
V-Vi4	25.60	67.84	3.2
V-Vi5	25.60	58.88	2.9

Abbreviation of wines: V-Vranec, Vi-Vinalco yeast, L-Lallemand yeasts: 1-Clos, 2-RC212, 3-D254, 4-BDX

4. Conclusion

Titrimetric methods for determination of SO_2 (free and total) and reducing sugars in wines were checked. Validation parameters of the methods confirmed that they are fast, accurate, precise and easily available in every laboratory. These methods are applicable in wineries for control of the content of SO_2 and sugars during the wine production. The content of SO_2 is usually higher in white wines compared to red wines since white wines are easily oxidizable and therefore need higher dose of SO_2 for protection. Vranec wines fermented with different yeasts contained appropriate quantities of free and total SO_2, enough for their protection from oxidation. All wines were dry, containing low value of reducing sugars (< 5 g/L).

5. References

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