INVESTIGATION OF A NON THERMAL EFFECT OF MICROWAVE TREATMENT

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The aim of our experiments was to demonstrate the non-thermal effect of microwave treatment on *Saccharomyces cerevisiae* fermentation activity. A method was developed for studying the effects of various treatments in the course of must fermentation. The raw material (must) was treated in different ways: (i) heat transfer; (ii) microwave treatment; (iii) inoculation with yeast and (iv) their combinations. The results of the treatments were compared with respect to alcohol concentration, sugar content and acidity. The results proved that sugar content of the treated samples rapidly decreased compared to the control sample, and fermentation time was 40% shorter in the fastest case. These results can be explained by the yeast inoculation and microwave treatment. Due to non-thermal effects fermentation capacity increased by about 30%, while the energy consumption decreased.

Keywords: *microwave radiation, non-thermal effect, Saccharomyces cerevisiae, grape must, fermentation*

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The application and presence of different species of microorganisms (bacterium, yeasts, filamentous fungi) is well known during the production of alcoholic beverages (FARKAS et al., 2005). The role of yeasts in wine making (e.g. wine yeast *S. cerevisiae)* is different but they are important in the development of taste and aroma (PRETORIUS, 2000).

The main task of winemaking technology is to optimize the fermentation process in order to reach suitable production of wine (EPERJESI et al., 1998). Complex processes are taking place simultaneously during the fermentation of must, which could influence the process in different ways. During fermentation the emphasis is mostly to optimize the alcohol, sugar and acid content (PICKERING et al.; 1998; BIACS et al. 2010). However, controlled fermentation is a well-regulated process with the correct application necessary for influencing parameters (CALADO et al., 2002; SABLAYROLLES, 2009). The effect of fermentation activity can be reduced by heat treatment (microwave heat treatment, heat treated with water-bath (GÉCZI et al., 2013; KORZENSZKY & MOLNÁR, 2014a,b) and so on.

It is evident that microwaves (MW) cause different biological effects depending on field strength, frequencies, wave forms, modulation and duration of exposure (RAI et al., 1994a,b). There has been considerable controversy over the non-thermal effect of MW radiation (DREYFUSS & CHIPLEY, 1980; WELT et al., 1994; WAYLAND et al., 1977; KOTHARI et al., 2011; TRIVEDI et al., 2011). Our aim was to backed up the non-thermal effects of MW, based on i.a. SHU-WEI et al. (2014) work.

During the measurements the MW radiation effect on yeast are significant. GRUNDLER and co-workers (1977, 1982, 1988) observed that the growth rate of yeast *S. cerevisiae* could either be increased up to 15% or decreased up to 29% by MW irradiation within 41.8–42.0 GHz range. Significant MW effect on synchronization of *S. carlsbergensis* yeast cells were observed by GOLANT and co-workers (1994). Exposure to MW radiation at 30 $\mu W/cm^2$ and 46 GHz induced synchronization as measured by cell density and bud formation. Authors

assumed that MW radiation activated cell-to-cell interaction resulting in the observed synchronization. BESZÉDES and co-workeres (2011, 2014) determined that applying MW pre-treatment, the volume of produced biogas from dairy and meat industry sludge was 19-times and 1.2-times higher respectively, than that of obtained from raw sludge. Based on this studies the MW pre-treatment positive potential to exert of grape must fermentation process was proved.

The aim of our experiments was the verification of the non-thermal effect of MW during grape must fermentation process.

1. Materials and methods

In our study grape must (from local vineyard) fermentation process was measured. The experiments were performed with two series of measurements in year 2010-2011. In the first experimental set (2010) the fermentation of four samples were compared. In the case of the control sample no treatment was used. Yeast (*S. cerevisiae,* IOC B 2000 active dried yeast) was added to the second sample. The third sample was treated with MW 2.45 GHz (50 W, 45 min, 32 °C, MARS5 MW Digestion System). In the case of the fourth sample a combined treatment (yeast+MW) was applied.

During the experiments the alcohol content was determined by the standard Malligand-device with an accuracy of ±0,2%, V/V (HUNGARIAN STANDARD, 1982; THÉNARD, 1875;), the sugar content of must was measured by NIR method using spectrophotometer type U-2910 HITACHI (NOVALES et al., 2009), and acidity by titration (OIV, 2009). Moreover, we investigated the energetic aspect of MW owen ad hot plate with Energy Logger 4000 power-meter (Conrad), with three repetitions. The initial sugar content was 179,2 g/l^{-1}.

In the second measurement series (2011) fermentation was compared applying six different treatments: (i) no treatment on control samples; (ii) hot plate heated (630 W, 45 min, 32 °C, YELLOW line, MST basic C); (iii) microwave-treated (50 W, 45 min, 32 °C); (iv) yeast supplementation (*S. cerevisiae*); (v) yeast inoculation while hot plate heated (32 °C); and (vi) microwave treatment and yeast supplement. The quantity of simultaneously treated sample was 525 ml. Due to the design of microwave resonator is the penetration depth was 100%. During the MW treatments was the temperature change detected with fiber optic temperature sensor (Probe, RTP-300Plus). After treatments the must fermentation was carried out at 15-16 °C (Minifors S-000113794) in these experiments.

The results were evaluated with MS Office Excel 2010 and TableCurve 2D. During the statistical analysis Anova and Student's T-test were used. The results shown in Table 3 and 4 were evaluated by ranking method (related rank numbers), where the same data received the same rank values.

2. Results and discussion

Based on references (e.g. SHU-WEI et al., 2014) our results support what we expected that the low power MW radiation has beneficial effect on yeast growth, so the fermentation also. The difference between untreated and treated samples was already seen at the beginning of the fermentation process. The sugar content of the control samples was decreased at a slower rate compared to the treated ones. Based on these results it can be stated that fermentation is significantly influenced by the treatments.

Fig. 1 shows that samples treated with yeast+MW supplementation reached the lowest value of sugar content on the 16^{th} day of fermentation. In the sample having only yeast supplementation the sugar content decreased faster than the control. The yeast inoculated

sample reached the minimum value on the 20th day of fermentation, while in the MW treated sample this phenomenon occurred only on the 24th day. The control sample reached the minimum value of sugar content (39 g/l^{-1}) on the 28th day of fermentation (end point).

Fig. 1.

The alcohol content (*Fig. 2*) of the control samples increased slower than in treated samples. Furthermore, the control sample gained alcohol content (11.6%) at the end of the fermentation process.

Samples treated with yeast+MW and inoculated only with yeast samples reached the highest alcohol content (12.6%, and 12.2% respectively) on the 20th day of fermentation, which implies that the treatment significantly influenced the speed of fermentation.

The yeast+MW treated sample achieved the highest alcohol content between the 24 and 28 days of fermentation (12.1-12.2%).

Fig 2.

At the beginning of fermentation acidity increased for a while and then decreased, as shown in other studies, too (KÁLLAY, 2010). This can be also clearly seen in our measurements (*Table 1*).

Table 1.

In the second replications (2011) we also carried out hotplate treatments, where the fermentation process advanced like in the first series of experiments. In the case of a second measurement series similar results were experienced with the sugar content as in the first measurement (*Fig. 3*). The combination treated (yeast+MW) sample reached the lowest value of sugar content the earliest on the 14th day of fermentation. It can be noted that yeast treated and hot-plate heated samples reached the lowest sugar content on the 16th day of fermentation (23 days total fermentation), while the remaining samples reached this more slowly.

Fig. 3.

Distinctly, fermentation started on the 2nd day of measurement. As shown in *Fig. 4* there was a significant difference between the alcohol content of the control sample (0.4%) and the treated samples (1 to 3.1%).

The alcohol contents of the combined treated samples reached the highest level (10.4% and 10.2%) on the 14th day of fermentation. These treatments also influence the speed of fermentation. The alcohol content of the must samples treated only with yeast inoculation or hot plate reached the highest level on the 18th day of fermentation (10% and 9.8% respectively).

Fig. 4.

Concerning acidity (*Table 2a-b*) it can be concluded that the combination treated samples have the largest acidity. The acidity change is not as uniform as the sugar and alcohol content change, because during fermentation yeast consumes some acids (tartaric acid, malic acid) while new ones also form (succinic acid, lactic acid) (EPERJESI et al., 1998).

It can be stated that the average acidity difference between Day 0 (must) and Day 23 (wine) was 23.31%. The difference between acidity was found to be 28.44%.

Table 2a-b

Table 3 and *4* show that in the biggest influence was found in case of yeast+MW treated samples. Yeast inoculated samples were in the second place, which means that after MW non-thermal effect the yeast influenced the treatments.

Table 3.

Table 4.

During the experiments we analysed the energetics aspects, too. The duration of the treatment were 45 min. Based on this measurements (using the power-meter) the MW used on average of 1109.52 kJ and hot plate used 204.14 kJ. The basic energy consumption (fan,

lights, rotating disc) of MW owen on average was 936 kJ. In order to clarify the energetic analysis additional tests are needed.

3. Conclusions

In the measurement series carried out in 2010 (control, yeast inoculated, MW treated, yeast+MW combined samples) and 2011 (control, yeast inoculated, hot plate heated, MW treated, yeast+MW and hot plate+yeast combined samples) gave similar results of fermentation process. The sugar content of the treated samples rapidly decreased compared to the control sample and the fermentation time was shorter by 40% in the fastest case. These results can be explained by the yeast inoculation and the MW treatment.

The statistical analysis showed no significant difference (p=5%) between each sample on the first series. In this case the non-thermal effect of MW is not present or has no effect on the results. The second series of measurements did not show significant difference between each sample as regards to the alcohol content during the whole fermentation. In the first third of fermentation there was verifiable difference (p=5%) between the samples.

It was concluded that a short-term heat treatment prior to fermentation until 32 ^{o}C influences the parameters of the fermentation in a positive way by using yeast. The fermentation time was reduced while the alcohol yield increased.

In aspect of energetics it can be stated that in case of hot plate treatment we need 5.4 times more energy that MW treatment, however the fermentation time increased in case of MW treatment 14.2% compared to hot plate heated treatments (*Fig. 4*).

In case of energetic aspect cooling reverse energy could be reduced. Due to non-thermal effects increased by the fermentation capacity about 30%.

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We wish to thank to “OTKA” (Hungarian Scientific Research Fund), “Bolyai Janos” Research Scholarship, TÁMOP-4.2.2.A-11/1/KONV and GOP-1.1.1-11.-2012-0157 projects.

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Table 1. Change of acidity (g/l) of the must during the fermentation of the control, yeast inoculated, microwave-treated and microwave and yeast treated samples.

	4th Day	8th Day	12th Day	16th Day	20th Day	24th Day	28th Day
Control	5.1±0.03	6.1±0.02	6.0±0.03	6.3±0.03	6.3±0.06	6.3±0.06	6.25±0.04
Yeast	5.5±0.02	6.2±0.03	6.1±0.03	5.6±0.06	5.5±0.06	5.55±0.06	5.45±0.03
Microwave	5.4±0.02	6.2±0.03	6.6±0.03	6.4±0.03	6.4±0.06	6.4±0.02	6.15±0.02
Microwave+ yeast	5.8±0.02	6.1±0.03	5.9±0.03	5.6±0.06	5.6±0.05	5.45±0.05	5.2±0.02

Table 2a.-b. Change of acidity (g/l) of the must during the fermentation of the control, hot plate heated, the microwave-treated, yeast inoculated, hot plate+yeast, and with microwave and yeast treated samples.

a.

	0th Day	1th Day	2th Day	3th Day	5th Day	7th Day
Control	4.3±0.03	4.55±0.02	4.33±0.02	4.97±0.04	5.45±0.04	5.52±0.02
Hot plate		4.48±0.02	4.72±0.06	5.38±0.02	5.62±0.03	5.35±0.02
Microwave		4.45±0.06	5.13±0.04	5.63±0.03	6±0.02	5.98±0.04
Yeast		4.47±0.06	4.87±0.06	5.8±0.04	5.62±0.04	5.37±0.03
Hot plate+yeast		4.22±0.02	5.28±0.02	5.97±0.06	6.12±0.03	5.4±0.06
Microwave+yeast		4.72±0.04	5.53±0.02	5.75±0.03	5.73±0.06	5.58±0.04

b.

	9th Day	12th Day	14th Day	16th Day	19th Day	23th Day
Control	5.47±0.04	5.37±0.02	5.39±0.02	5.37±0.03	5.73±0.04	5.63±0.04
Hot plate	5.72±0.02	5.82±0.04	5.77±0.02	5.82±0.02	5.88±0.03	5.62±0.03
Microwave	5.78±0.06	5.78±0.03	5.68±0.04	5.78±0.06	5.72±0.06	5.82±0.02
Yeast	5.05±0.04	5.35±0.04	5.05±0.04	5.35±0.03	5.28±0.06	5.22±0.04
Hot plate+yeast	5.52±0.02	5.63±0.02	5.38±0.04	5.63±0.04	5.22±0.04	5.52±0.03
Microwave+yeast	5.35±0.02	5.38±0.04	5.38±0.02	5.38±0.03	5.17±0.03	5.35±0.06

Table 3. The effect of treatments on different parameters

(Rating between 1-4; 1 – minimum impact, 4 – maximum impact)

	Sugar Content (7^{th} Day)	Alcohol Formation Rate	A Final Alcohol Content	Summ. Total Influence
Control	1	1	1	3
Microwaves	2	2	3	7
Yeast	3	3	2	8
Yeast and microwave	4	4	4	12

Table 4. The effect of treatments on different parameters

(Rating between 1-6; 1 – minimum impact, 6 – maximum impact)

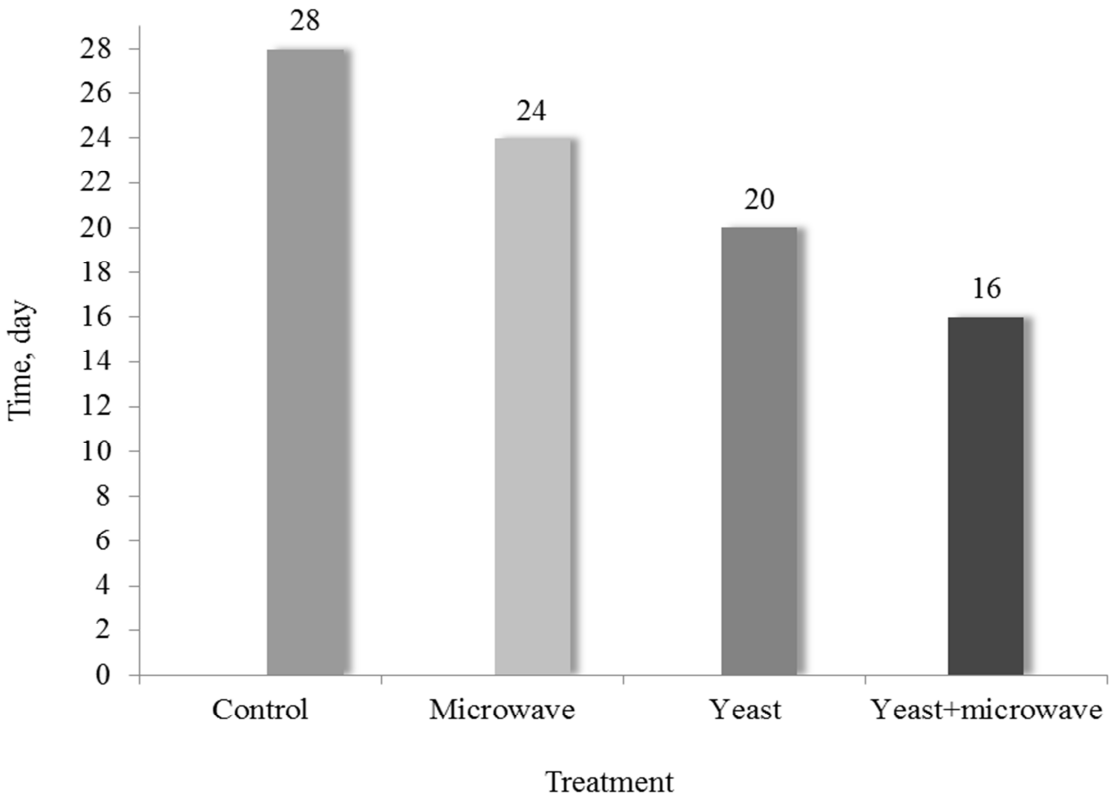
	Sugar Content (7^{th} Day)	Alcohol Formation Rate	A Final Alcohol Content	Summ. Total Influence
Control	2	1	3	6
Hot plate	4	3	3	10
Microwaves	3	2	1	6
Yeast	6	4	3	13
Hot plate + yeast	1	5	5	11
Yeast and microwave	5	6	6	18

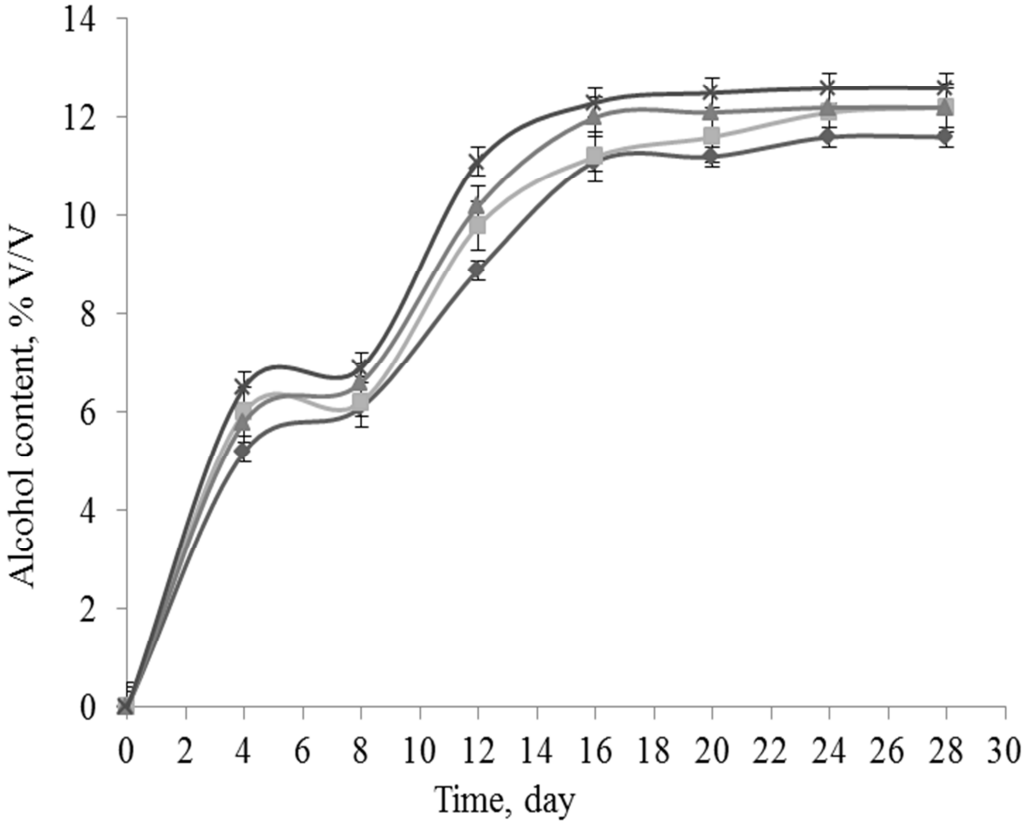
Fig. 1. Achieving final sugar content (39 g/l^{-1}) during the fermentation due to different treatments shown in days.

Fig. 2. Changes of the alcohol content of the must during the fermentation of the control ( ), the microwave ( ), the yeast ( ) and the yeast and microwave treated ( ) samples.

Fig. 3. Achieving final sugar content (39 g/l^{-1}) during the fermentation due to different treatments shown in days.

Fig. 4. Change of the alcohol content of the must during the fermentation of the control ( ), the hot plate heated ( ), the microwave-treated ( ), the yeast inoculated ( ), the hot plate + yeast ( ), and with microwave and yeast ( ) treated samples.




Alcohol content, % V/V
Time, day
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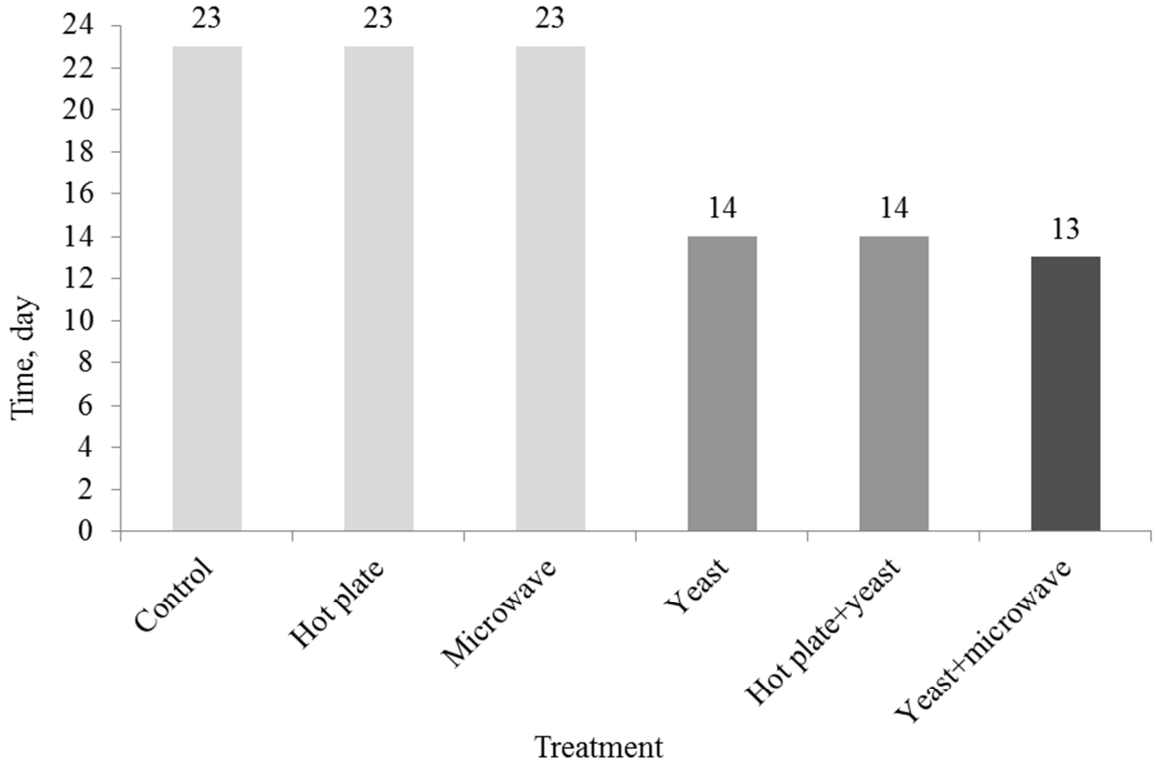
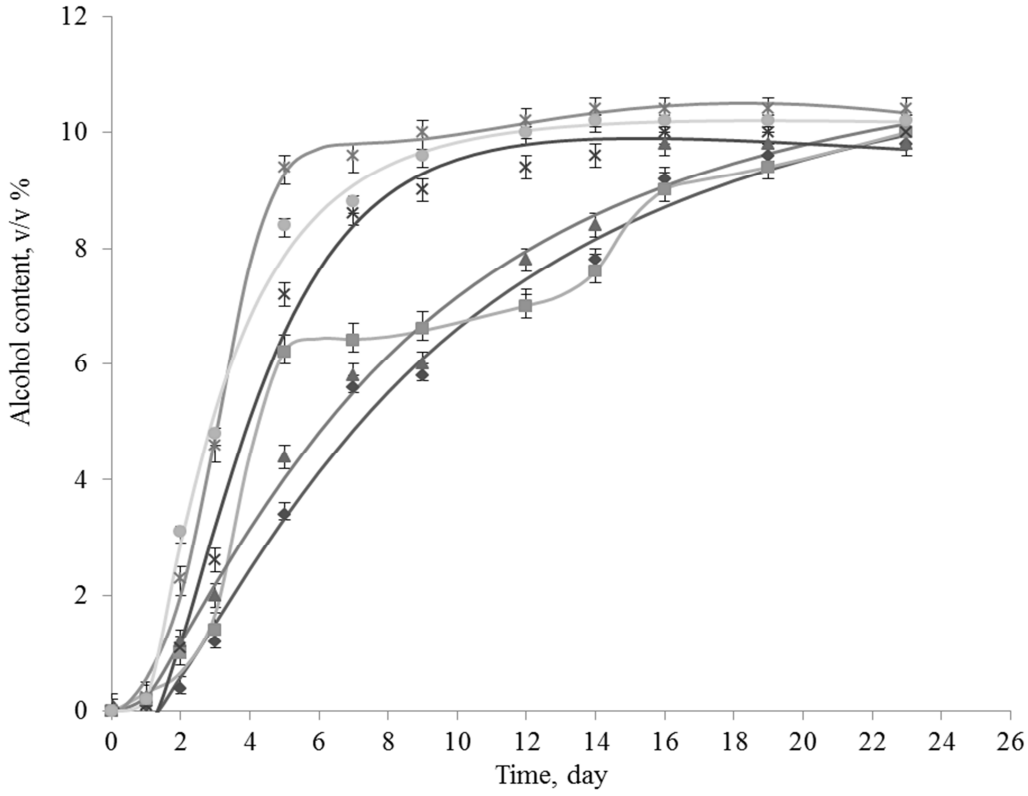
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30


Time, day
Treatment
23
23
23
14
14
13
Control
Hot plate
Microwave
Yeast
Hot plate+yeast
Yeast+microwave


Alcohol content, v/v %
Time, day
0
2
4
6
8
10
12
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18
20
22
24
26