

**THESES OF DOCTORAL (PhD) DISSERTATION**

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**MOSONMAGYARÓVÁR  
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**THESES OF DOCTORAL (PhD) DISSERTATION**

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**EFFECT OF MICROWAVE TREATMENT OF *SACCHAROMYCES  
CEREVISIAE* LIVING ACTIVITIES, GRAPE MUST  
FERMENTATION AND CELLULOSE BREAKDOWN PROCESS**

Written by:  
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## **1. RESEARCH TARGETS**

During the research, first the effect of 2,45 GHz microwave radiation on living cells and enzymes was investigated. The degree of the non-thermic effect of the microwave radiation was, first of all, tried to detect and determine through the treatment of *Saccharomyces cerevisiae* during the measurements. The research work was intended to decide whether microwave radiation has affect on proliferation and fermentation characteristics of the cells. The use of microwave steam explosion pretreatment and enzymatic hydrolysis was also investigated. It was taken is consideration, too, to fulfil enzyme-activity measurements in order to facilitate of further developments at various industrial branches (e. g. bioethanol production), as well.

## **2. MATERIALS AND METHODS**

### **2.1. Microwave treatment**

During our research, the effect of microwave radiation on living cells (*Saccharomyces cerevisiae*) was investigated. For the determinations a model MARS microwave reaction system (digestion equipment) was used which is suitable for both the treatment of solid and liquid samples.

## **2.2. Investigations on destruction and proliferation of *Saccharomyces cerevisiae***

During the research the yeast *Saccharomyces cerevisiae* was affected by radiation in microwave region, and then its destruction and proliferation features were investigated. After applying microwave treatments of various electric powers (50, 100, 400, 600 and 900 W) and treatment times (30, 60, 90 and 120 minutes) used, destruction diagrams were determined. For the determination of proliferation diagrams, electric powers of 50 and 400 W were applied at 37 °C temperature for 60 minute radiation time. Proliferation intensity was followed by optical density measurements.

## **2.3. Glucose decomposition investigations**

Measurements for glucose decomposition of yeast were also fulfilled; the experiments were made in a laboratory fermenter and controlled fermentation equipment, as well. During the measurements, 50 W electric power was applied for 60 minute treatment time, at maximum temperature of 37 °C. Untreated control sample was used for comparison purposes. Glucose decrease was determined by spectrophotometric analysis.

## **2.4. Investigations of the fermentation processes in grape must**

During the microwave treatment of grape-must, 50 W electric power was used for 45 minute treatment time at 37 °C temperature. These treatment parameters were chosen in order to be able to investigate the results under realistic circumstances, and to confirm the non-thermal effect of low-power microwave radiation. 3 series of grape-must fermentation were set with different treatments each. Control (untreated), inoculated with

yeast only, treated with microwave radiation only, and combined (yeast and microwave) samples were used during the first series of investigation. During the second investigation series, treatments on traditional cooking-plates were also made. In this second series, control (untreated), inoculated with yeast only, traditionally treated only, treated with microwave irradiation only, inoculated with yeast and microwave treated, and inoculated with yeast and traditionally treated samples were used. During the third series frozen samples were used. During the fermentation process, determinations of alcohol, sugar and acid content were made.

## **2.5. Microwave explosion pretreatment and enzymatic decomposition of vine-branches**

During the decomposition of cellulose from vine-branches, physical-chemical treatments (i. e. steam explosion pretreatment, autoclave treatment, traditional treatment) were applied; and all the above with samples of added enzymes. The process of cellulose decomposition was followed by detecting sugar content increase.

First, preliminary experiments were made during the investigations with microwave steam explosion pretreatment for determining glucose yield at various pressure values (1; 1.5; 2; 5; 11; 15; and 25 bar). Besides the treatments, cellulose degrading enzyme (from *Trichoderma reesei*) was given to untreated sample for the control sample. During the treatment of vine-branches, microwave electric power was set to 1600 W, treatment time to 5 minutes, maximum temperature to 150 °C. 20 g from the shredded vine-branch (10 g for each pot) was placed into the teflon-pot of the MARS device used for microwave in steam explosion pretreatment, and 70 ml of Na-acetate-acetic acid buffer solution was added.

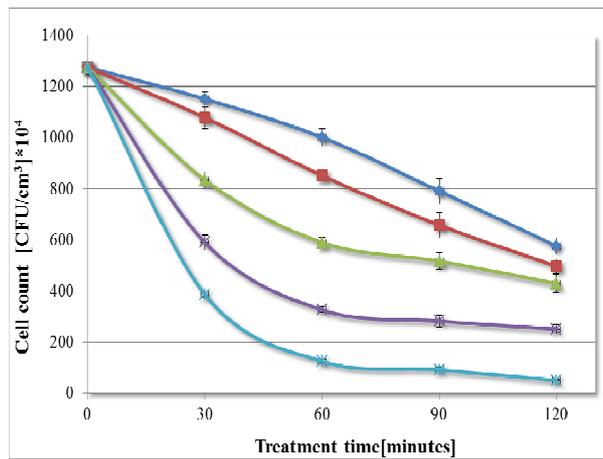
Besides the changing of the treatment parameters, various experimental patterns were applied: (1) steam explosion pretreatment through microwave energy application to the vine-branch, and addition of untreated enzyme; (2) steam explosion pretreatment through microwave energy application to the vine-branch, and addition of microwave treated enzyme; (3) autoclave pretreatment of the vine-branch, and addition of untreated enzyme; and (4) traditional treatment of the vine-branch, and addition of untreated enzyme.

### **3. RESULTS**

#### **3.1. Results of investigations with radiation of *Saccharomyces cerevisiae***

##### *3.1.1. Cell destruction results*

From the decomposition intensity of yeast treated with 50 W electric power it can be seen that decomposition intensity is proportionally increased in dependence on different treatment times. Using this power level, yields were not fully destructed even by a 120 minute radiation (*Figure 1*).

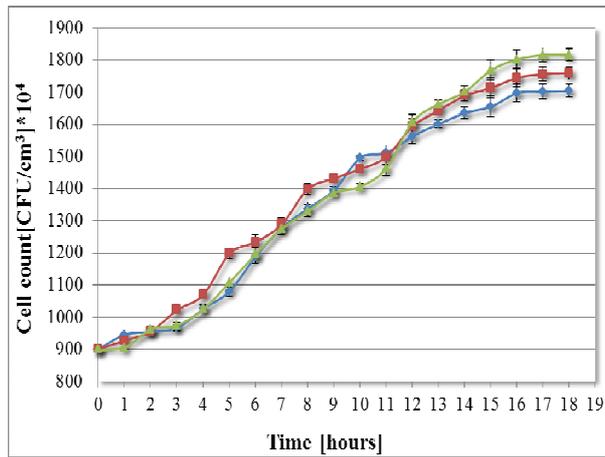


**Figure 1:** Destruction curve of *Saccharomyces cerevisiae* using microwave radiation of 50 (◆), 100 (■), 400 (▲), 600 (✕), and 900 W (✱)

The results show the shorter the sample treatment time the greater the difference among destruction rates. The longer the microwave radiation is used greater the decrease in these differences. This leads to the consequence that it is not worth treating the yeast at elevated electric power levels owing to the high destruction and decomposition rates, although a microwave treatment at lower temperatures and electric power levels is more favourable for the yeasts.

### 3.1.2. Investigations of proliferation

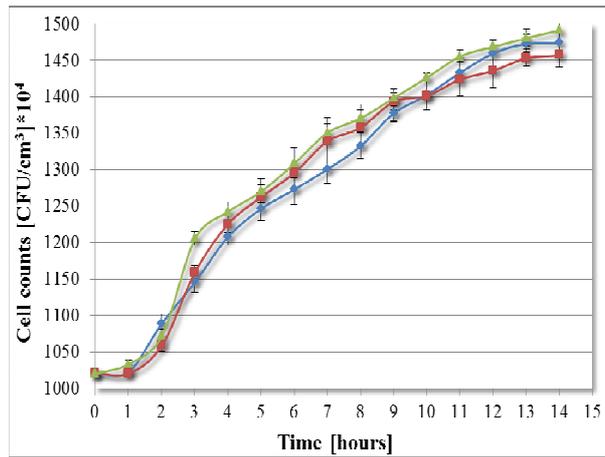
Besides destruction investigations, measurements were made to determine the proliferation characteristics of *Saccharomyces cerevisiae*, as well. During these proliferation experiments, first the proliferation curves of the yeast were determined, then the proliferations in various samples were compared to each other (Figure 2). Microwave electric power was 400 W, treatment time 60 minutes, and final temperature set in the microwave device was 37 °C.



**Figure 2:** Proliferation curves of *Saccharomyces cerevisiae* as the effect of various treatments for control samples (—◆—), treated water samples (—■—), and samples treated together (—▲—)

From the results it can be seen that there are no significant differences among the latency, the accelerating and the exponential sections. The greatest difference was observed in the case of the control sample which alters from those of the other 2 samples after the 13<sup>th</sup> hour of the measurement time. This difference is even more obvious from the 14<sup>th</sup> hour of the measurement. The shape of the curve is approximately the same, however it can be stated that there are differences among the final cell counts regarding the different treatments. This difference is the greatest in the case of the sample where water was treated together with the yeast.

In the following measurements the proliferation curves of control samples, traditionally treated samples, and microwave treated samples were determined. From *Figure 3* can be seen that there were no significant differences regarding the microwave treated sample value (50 W electric power) and those of the other the samples.

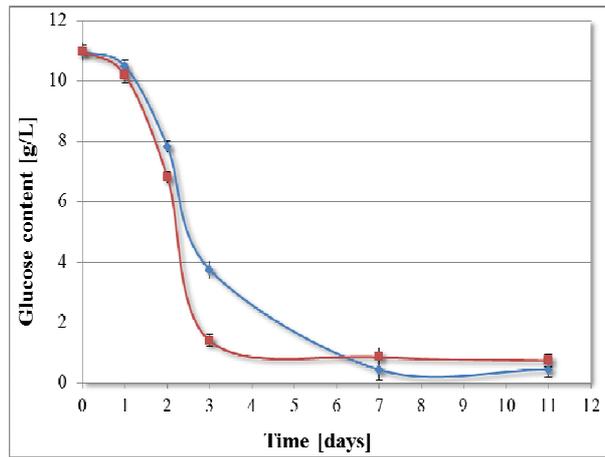


**Figure 3:** Proliferation curves of *Saccharomyces cerevisiae* as the effect of various treatments for control samples (—◆—), traditionally treated samples (—■—), and microwave treated samples (—▲—)

Based on the results of proliferation experiments it can be stated that microwave radiation treatment affects the life cycle of yeasts in such a way that greater final cell counts can be observed at the end of the proliferation period as effect of the treatments.

### 3.1.3. Glucose degradation experiments

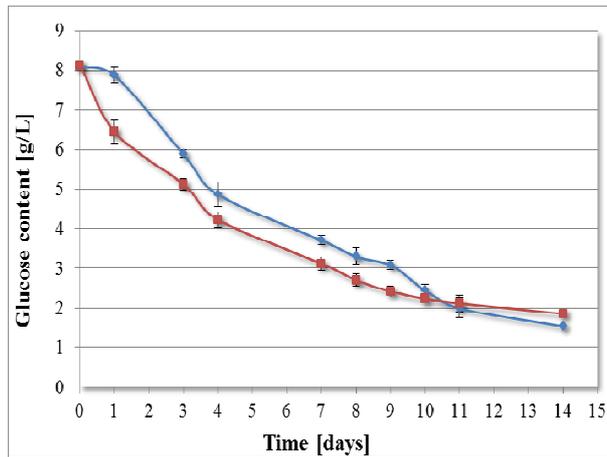
Following the determination of the proliferation curves experiments were designed to measure the glucose degradation activity of the yeast. On *Figure 4* fermentation activities of control samples and microwave treated samples are compared using MINIFORS fermenter.



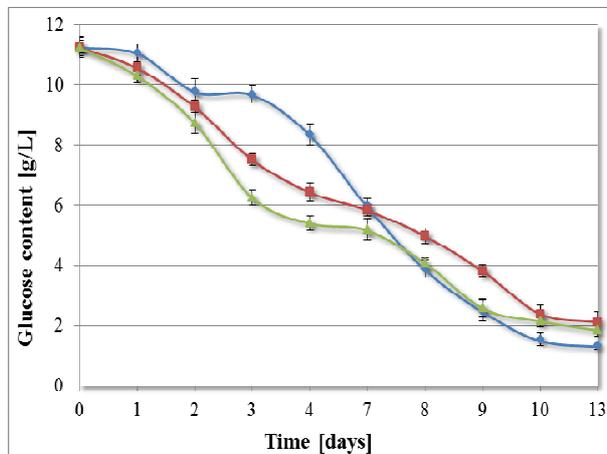
**Figure 4:** Changes in fermentation activity of yeast; control sample (—◆—), microwave treated sample (—■—)

On the basis of the result it can be stated that the sugar content of microwave treated samples decreased more quickly which means the activity of the yeast was greater. Therefore, it can be concluded that low-energy microwave radiation has an effect on fermentation activity of yeasts. The fermentation process is the most intensive during the first phase of fermentation - this is valid for the control sample, however, in case of treated samples it takes longer time.

After the experiments made in Minifors fermenter, the examinations were made in the great fermenter, too (*Figure 5*), with the alteration that in this case the microwave treatment time was 7 hours, and the volumes of both the control sample and the treated sample amounted 70 litres. Finally, both methods were compared (*Figure 6*).



**Figure 5:** Changes in fermentation activity of yeast; control sample (—◆—), microwave treated sample (—■—)

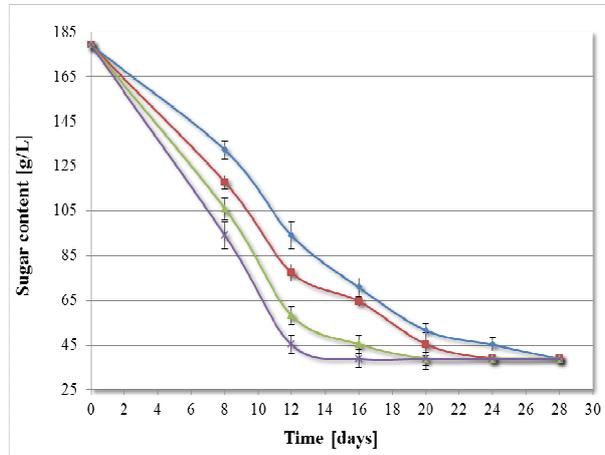


**Figure 6:** Changes in fermentation activity of yeast; control sample (—◆—), sample treated prior to fermentation (—▲—), and sample treated continuously (—■—)

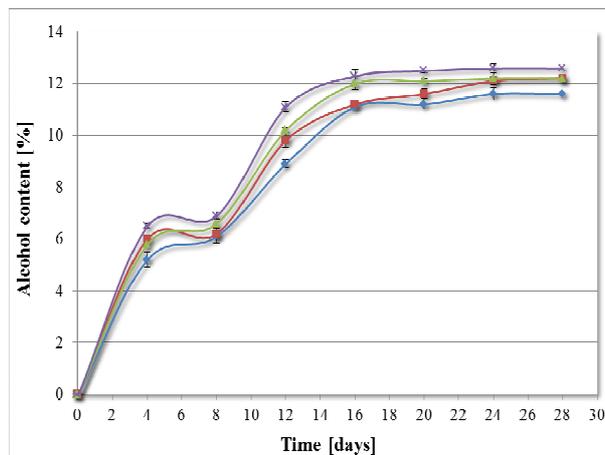
Similar results were obtained during all the three series of experimental measurements. In the first phase of the fermentation process sugar decrease of the control sample was less intensive than that of the microwave treated samples. In the third last phase of the fermentation sugar contents of the samples were nearly equal, however, it can be observed that the final results of the control sample are the lowest ones.

### 3.2. Effect of microwave treatment on the fermentation of grape-must

For the microwave treatment of grape-must, 50 W electric power, 45 minutes of treatment time, and 37 °C temperature were applied.

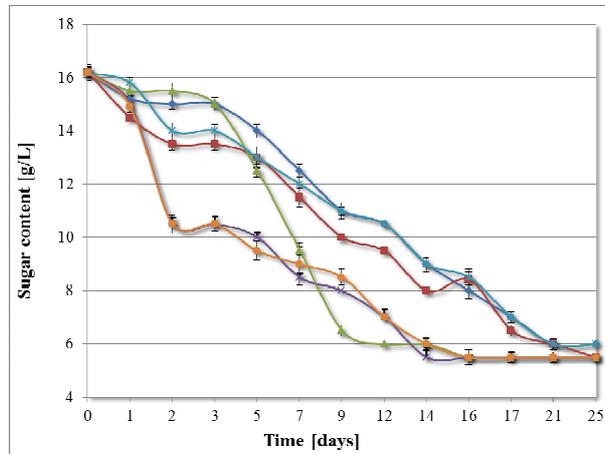


**Figure 7:** Changes in sugar content of grape-must during the fermentation process; control sample (—◆—), microwave treated sample (—■—), sample inoculated with yeast (—▲—), and microwave treated sample inoculated with yeast (—×—).

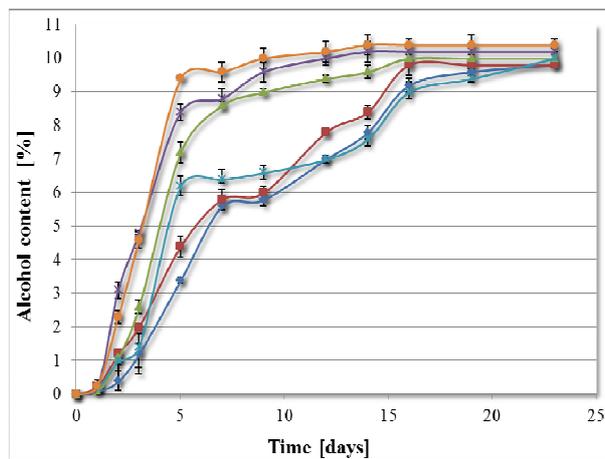


**Figure 8:** Changes in alcohol content of grape-must during the fermentation process; control sample (—◆—), microwave treated sample (—■—), sample inoculated with yeast (—▲—), and microwave treated sample inoculated with yeast (—×—)

In the second series of measurements (*Figure 9*) the samples were expanded with traditional heat treatment (cooking plate) measurements in order to exclude any eventual thermal effect caused by microwave radiation.



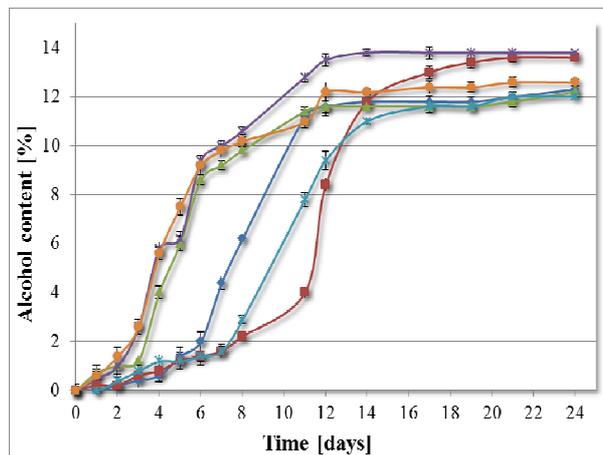
**Figure 9:** Changes in sugar content of grape-must during the fermentation process; control sample (◆), microwave treated sample (■), sample inoculated with yeast (▲), microwave treated sample inoculated with yeast (×), traditionally treated sample (✱), and traditionally treated sample inoculated with yeast (●)



**Figure 10:** Changes in alcohol content of grape-must during the fermentation process; control sample (◆), microwave treated sample (■), sample inoculated with yeast (▲), microwave treated sample inoculated with yeast (×), traditionally treated sample (✱), and traditionally treated sample inoculated with yeast (●)

On the basis of the results it can be stated that both measurement series resulted in similar results. Sugar content was more quickly decreased in the samples, and fermentation times were only shortened by 40% in the best case. These are most probably due to the inoculation with yeast, and the minimal heat effect caused by the treatments. It can be told that a short heat treatment at maximum 37 °C temperature, with added selected yeast prior to fermentation affects positively the fermentation parameters; fermentation time becomes shorter while alcohol yield is growing.

In the case of the third grape-must fermentation experiment, detecting the alcohol content was emphasized. The change of alcohol content during the fermentation process was measured depending on various treatments. The difference was during this series of measurement that frozen grape-must samples were used (*Figure 11*).

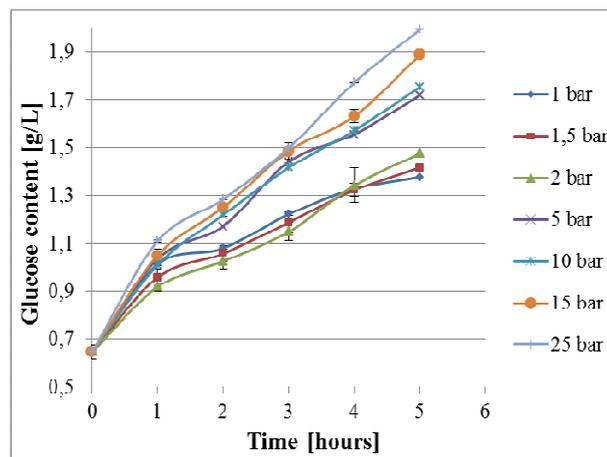


**Figure 11:** Changes in alcohol content of frozen grape-must during the fermentation process; control sample (◆), microwave treated sample (■), sample inoculated with yeast (▲), microwave treated sample inoculated with yeast (×), traditionally treated sample (∗), and traditionally treated sample inoculated with yeast (●)

The fermentation process itself can be considered remaining the same as in the case of sample treated only with yeast, however, the alcohol content was the lowest among the samples inoculated with yeast. The three samples treated with non-combined treatments (i. e. control sample, traditionally treated sample and microwave treated sample) show an interesting observation regarding fermentation course. The fermentation starts very slowly. The reason is that owing to freezing, there needed more time for the regeneration of in grape-must naturally occurring wild yeasts.

### 3.3. Results of microwave and enzymatic treatments of vine-branches

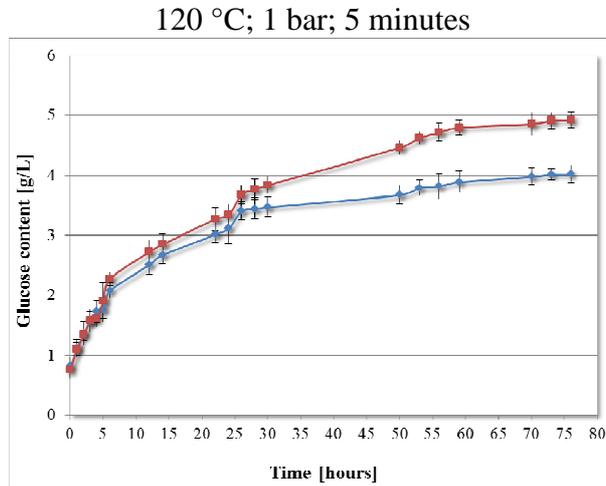
Preliminary experiments were made for microwave treatment (1600 W, 5 minutes) and enzymatic degradation of vine-branches (*Figure 12*), where the amount of glucose produced was measured (for 5 hours) after treatments at different pressures (1; 1.5; 2; 5; 10; 15; and 25 bar).



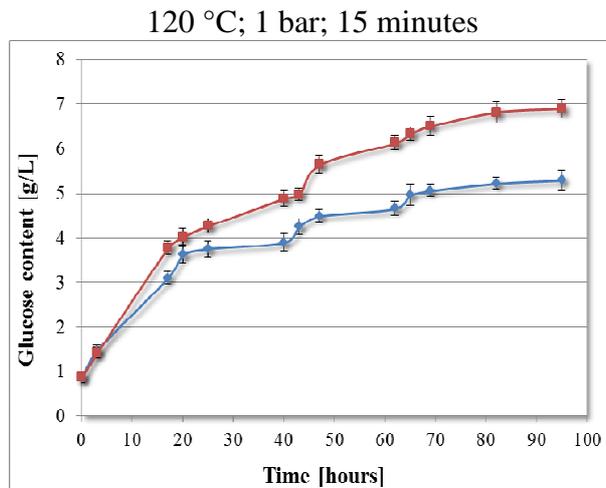
**Figure 12:** Amount of glucose derived from vine-branches treated at various pressures

After preliminary experiments, measurements were made to follow the process of cellulose degradation not only for 5 hours but till the time no further glucose production was observed. Besides the microwave pre-

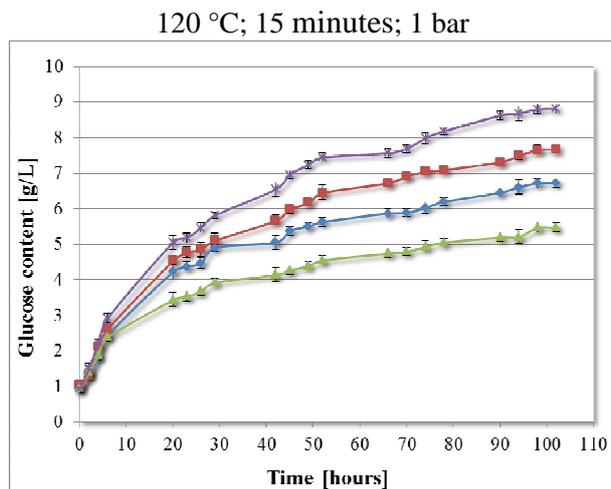
treatments, autoclave treatments and traditional treatments were also used. Another modification was that (beside the vine-branch treatments) the enzyme used was microwave-treated in certain cases. Different treatments were thereby developed for the measurements through variations in microwave electric power, temperature, pressure, and treatment time.



**Figure 13:** Enzymatic degradation of vine-branches; autoclave treatment 430 W (—▲—); microwave treatment 400 W (—■—)



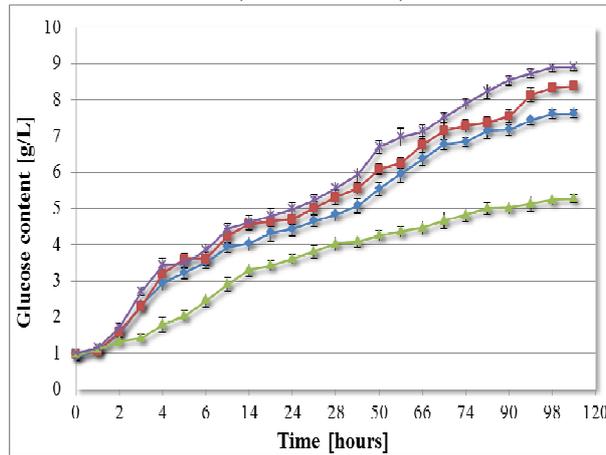
**Figure 14:** Enzymatic degradation of vine-branches; microwave treatment (400 W) of the branches and untreated enzyme (—▲—); microwave treatment (400 W) of the branches and treated enzyme (—■—)



**Figure 15:** Enzymatic degradation of vine-branches at various microwave electric power values; traditional treatment of the branches and untreated enzyme (—▲—); autoclave treatment of the branches and untreated enzyme (—◆—); microwave treatment of the branches and untreated enzyme (—■—); microwave treatment of the branches and treated enzyme (—×—)

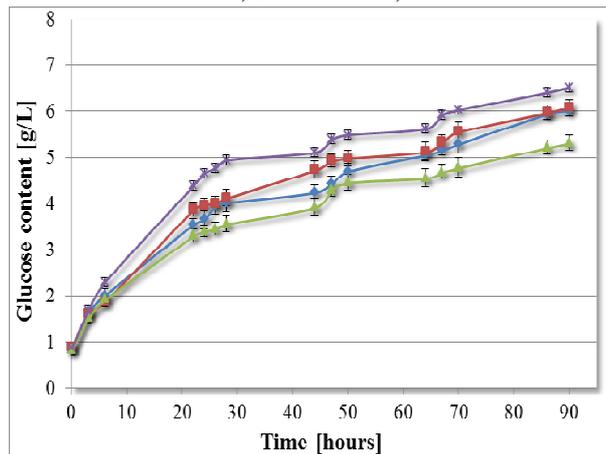
The results definitely show that microwave radiation affects the activity and cellulose degradation ability of the enzyme. In this experiment (*Figure 15*) the autoclave worked at 430 W electrical power, while those of the microwave radiation was 1600 W therefore the microwave electrical power had to be modified (*Figure 16*).

120 °C; 15 minutes; 1 bar



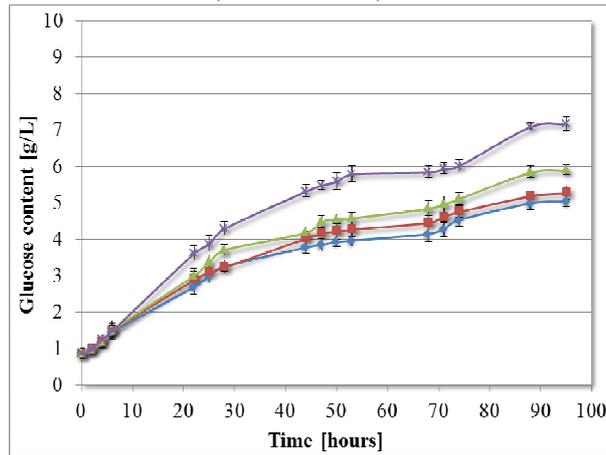
**Figure 16:** Enzymatic degradation of vine-branches at 440 W microwave electric power values during 4 different treatments; traditional treatment of the branches and untreated enzyme (—▲—); autoclave treatment of the branches and untreated enzyme (—◆—); microwave treatment of the branches and untreated enzyme (—■—); microwave treatment of the branches and treated enzyme (—×—)

120 °C; 30 minutes; 1 bar



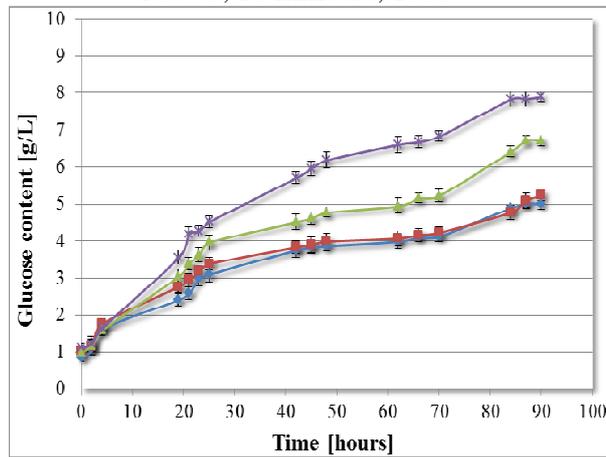
**Figure 17:** Enzymatic degradation of vine-branches at 440 W microwave electric power values during 4 different treatments; traditional treatment of the branches and untreated enzyme (—▲—); autoclave treatment of the branches and untreated enzyme (—◆—); microwave treatment of the branches and untreated enzyme (—■—); microwave treatment of the branches and treated enzyme (—×—)

150 °C; 10 minutes; 1600 W

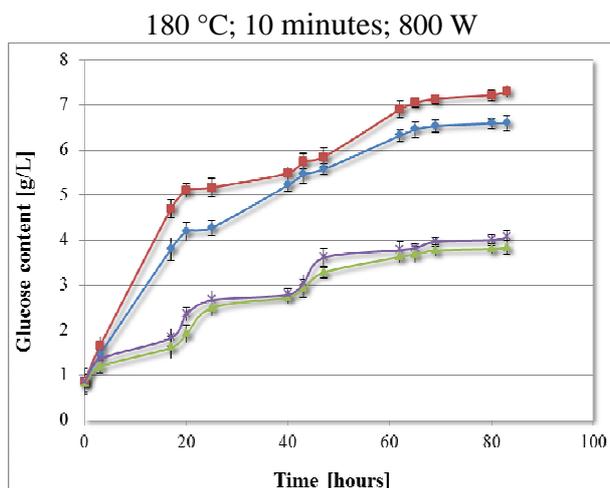


**Figure 18:** Results of the experiments; after treatments at 2 bar: microwave treatment and untreated enzyme (◆); microwave treatment and treated enzyme (■); after treatments at 5 bar: microwave treatment and untreated enzyme (▲); microwave treatment and treated enzyme (✕)

180 °C; 10 minutes; 1600 W



**Figure 19:** Results of the experiments; after treatments at 2 bar: microwave treatment and untreated enzyme (◆); microwave treatment and treated enzyme (■); after treatments at 5 bar: microwave treatment and untreated enzyme (▲); microwave treatment and treated enzyme (✕)



**Figure 20:** Glucose yields from vine-branch experiments; microwave treatment at 1 bar and untreated enzyme (—■—); microwave treatment at 2 bar and untreated enzyme (—●—); microwave treatment at 1 bar and treated enzyme (—×—); microwave treatment at 2 bar and treated enzyme (—■—)

Based on the results it can be told that significant differences regarding glucose yield can be caused through varying treatment time, microwave electrical power and temperature. The efficacy of the hydrolysis process was affected by modifying these above mentioned parameters. This value can even further be increased if the enzyme used for the hydrolysis should also be microwave pre-treated which obviously fortifies the enzyme activity can be increased by radiation. During the experiments with untreated and treated enzymes, differences of minimum 1.1%, and maximum 22.1% were observed. Another dependence is that a treatment of 30 minutes is too much during the treatment of both the vine-branches and the enzyme since there was no greater difference among the samples (treated and untreated enzyme: difference of 6.8%). Making comparisons among the treatments at 2 bar and 5 bar pressure at 150 °C and 180 °C temperatures it can be determined that greater percentage differences were

obtained at the end of the hydrolysis when using treatments at lower temperatures.

#### **4. NEW SCIENTIFIC RESULTS (THESES)**

- 1. I have confirmed that the degradation intensity of *Saccharomyces cerevisiae* is significantly increased depending on the time, between 30 and 120 minutes treatment time, using microwave radiation of 2,45 GHz frequency and electrical power of 50-900 W (0,095-0,19 W/cm<sup>3</sup>).**
- 2. A microwave radiation at maximum 37 °C temperature for 45-60 minutes with 50 W electrical power increases the growth of *Saccharomyces cerevisiae*, as well as its glucose fermentation activity and grape-must fermenting ability thereby having the consequence of significant decrease in time needed for the alcoholic fermentation of grape-must.**
- 3. An increased glucose yield can be achieved during the microwave steam explosion pretreatment of vine-branches if the cellulase enzyme from *Trichoderma reesei* [1,4-(1,3;1,4)- $\beta$ -D-glucane-4-glucanohydrolase] is also microwave treated. The enzyme activity is remarkably increased when using a microwave radiation treatment of 400-440 W electrical power for 15 minutes.**
- 4. I have confirmed that the treatment of vine-branches can be more effectively realized through steam explosion treatment to autoclave treatment when using the same steam explosion parameters (400-440 W, 120 °C, 5-30 minutes, 1 bar).**

## 5. PUBLICATIONS

### SCIENTIFIC PUBLICATIONS IN FOREIGN LANGUAGES

1. **Kapcsándi V., Kovács A.J., Neményi M., Lakatos E. (2015):** Investigation of a non thermal effect of microwave treatment, Acta Alimentaria 45 Vol. 3 (*in press*)

### SCIENTIFIC PUBLICATIONS IN HUNGARIAN LANGUAGE

1. **Lakatos E.; Kovács A. J.; Kapcsándi V.; Neményi M. (2012):** Alacsony teljesítményű mikrohullámú sugárzás hatása a cellobiáz enzim működésére, Acta Agronomica Óváriensis 54 (1), pp. 3-11.
2. **Kapcsándi V.; Lakatos E.; Fábry Zs. N.; Neményi M. (2012):** Narancslé mikrohullámú hőkezelése, Acta Agronomica Óváriensis 54 (2), pp. 39-44.
3. **Kapcsándi V.; Neményi M.; Lakatos E. (2013):** Alacsony teljesítményű mikrohullám hatása a must erjedésére. Review of Faculty of Engineering; Analecta Technica Szegedinensia. University of Szeged, Faculty of Engineering, pp. 73-78.; ISSN 1788-6392

### FULL-CONTENT PROCEEDINGS ON SCIENTIFIC CONFERENCES

1. **Kapcsándi, V.; Lakatos, E.; Kovács, A.J.; Neményi, M. (2012):** Alacsony teljesítményű mikrohullámú sugárzás hatása olaszrizling must erjedésére. (In: Kovácsné Gaál Katalin (ed.) XXXIV. Óvári Tudományos Nap: A magyar mezőgazdaság - lehetőségek, források, új gondolatok. Mosonmagyaróvár, 2012.10.05., pp. 62-67. ISBN:978-963-9883-93-2)

2. **Kapcsándi, V.; Neményi, M.; Lakatos, E. (2013):** Low-power microwave radiation effect of fermentation of grape must. Science for Sustainability. International Scientific Conference for PhD Students. University of West Hungary. Győr, March 19-20, 2013., pp. 361-365.
3. **Kapcsándi, V.; Neményi, M.; Lakatos, E. (2013):** Effect of microwave treatment of the grape must fermentation process. Food Science Conference 2013 – With research for the success of Darányi Program. Budapest. 2013. 7-8th 11., pp. 72-75. Full content on CD. ISBN: 978-963-503-550-2
4. **Kapcsándi, V.; Neményi, M.; Kovács, A. J.; Lakatos, E. (2014):** Frissen préselt és fagyasztott must alkoholtartalmának vizsgálata a fermentáció folyamán különböző kezelések esetén, In: XXXV. Óvári Tudományos Nap: A magyar és nemzetközi agrár- és élelmiszer-gazdaság lehetőségei, Mosonmagyaróvár, Nyugat-magyarországi Egyetem Mezőgazdaság- és Élelmiszertudományi Kar (2014.11.13), 2014., pp. 36-40. (ISBN:978-963-334-193-3)

#### **ABSTRACTS PUBLISHED IN SCIENTIFIC CONFERENCE PROCEEDINGS**

1. **Lakatos, E.; Kovács, A.J.; Kapcsándi, V.; Neményi, M. (2015):** Influence of the cellobiase enzyme activity by microvawe radiation, 2<sup>nd</sup> International Conference on Food and Biosystems Engineering, 28-31 May 2015, Greek, Mykonos Island, p. 165 (Abstract)

#### **POPULAR SCIENTIFIC PUBLICATIONS**

1. **Kapcsándi, V. (2010):** A biogáztermelés alapjai. MezőHír. XV. No. 4, pp. 80-82.
2. **Kapcsándi, V. (2010):** Működő biogázüzemek Magyarországon. MezőHír. XV. No. 6, pp. 111-113.