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# ENZYMATICKÁ A MECHANICKÁ DISRUPČNÍ METÓDA RASOVÝCH CELULOTICKÝCH BUNKOVÝCH STIEN JAKO FAKTOR OVPLYVŇUJÚCI ICH *IN VITRO* STRÁVITEĽNOSŤ
# ENZYMATIC AND MECHANICAL DISRUPTION METHOD OF ALGAL CELLULOTIC CELL WALLS AS A FACTOR INFLUENCING THEIR *IN VITRO* DIGESTIBILITY

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**Abstract:** In this study it was evaluated the influence of mechanical and enzymatic disruption methods of algal cell walls on values of *in vitro* digestibility of green freshwater algae (*Chlorella kessleri*, *Scenedesmus quadricauda*, *Chlorella* sp.) and cyanobacterium *Spirulina platensis*. *In vitro* digestibility of dried matter and organic matter were determined by enzymatic-gravimetric method after pepsin, pancreatin and combined hydrolysis. The lowest values of digestibility were assessed in green alga *Sc. quadricauda*, other samples of green algae distinctly higher digestible. Cyanobacterium *Sp. platensis* was more digestible in comparison with investigated green algae, due to absence of cellulose in cyanobacterial cell wall. Mechanical and enzymatic disruption methods of cell walls of green algae resulted in significant rise in values of their digestibility, thereby increase the affect of utilization of nutrients from their biomass.
**Keywords:** cell wall, digestibility, disruption methods, green freshwater algae

## INTRODUCTION

Green freshwater algae and cyanobacterium *Spirulina platensis* (blue-green alga) contain broad spectrum of nutritious compounds like proteins, carbohydrates, lipids, vitamins, pigments and minerals. From this reason, algal biomass could be used as an alternative source of nutrition for human population, for enhancing the nutritional value of food and for the production of food supplements, animal feed additives, cosmetics, nutraceuticals and pharmaceuticals **(Zhou *et al*., 2005; Spolaore *et al*., 2006; Becker, 2007)**.

Gravimetric filtering *in vitro* method for determination of digestibility is comparatively fast and inexpensive, from this fact it can be widely used for routine analyses of nutrient digestibility **(Mišurcová, 2012).** The values of digestibility could be influenced by used method of digestibility determination, by variable structure of algal cell walls and further or by different cell shape.

Green freshwater algae contain cellulose in their cell walls. This situation causes a problem for human and non-ruminants because they absent enzyme cellulase and this fact leads to the limited availability of nutritional components from algal biomass. The cell wall of *Sp. platensis* is formed by four layers without cellulose, that consisted of peptidoglycan except the second one, that contains β-(1-2)-glucan, which is indigestible for human **(Ciferi, 1983).**

However, many studies have been published on nutrition composition of green freshwater algae, but there is still lack of information about determination of *in vitro* digestibility of green freshwater algae and further about methods leading to effective disruption of algal cellulotic cell walls.

The objectives of work were determine *in vitro* digestibility in green freshwater algae (*Chlorella kessleri*, *Scenedesmus quadricauda*, *Chlorella* sp.) and cyanobacterium *Spirulina platensis* by enzymatic-gravimetric method using Daisy incubator, further to performe disruption methods of algal cell walls by mechanical and enzymatic way and to evaluate the influence of these methods on their values of *in vitro* digestibility.

## MATERIAL AND METHODS

**Algal materials:** In solar photobioreactor cultivated species of green freshwater microalgae *Chlorella kessleri*, *Scenedesmus quadricauda* and cyanobacterium *Spirullina platensis* were obtained from Academic and University Center in Nové Hrady, CZ; in cascade-type cultivation autotrophically cultivated alga *Chlorella* sp. was obtained from Academy of Science in the CZ, Department of Phototrophic Microorganisms in Třeboň. All investigated samples were acquired in dried forms.

**Disruption methods of cell walls:** Two disruption methods by mechanical and enzymatic way were provided by using an oscillatory globe mill (MM 301; Retsch, Germany) and by using enzyme cellulase from *Trichoderma viride* (3-10 units/mg solids; Sigma-Aldrich).

*Disruption of algal cell walls by oscillatory globe mill*: Disruption of algal cell walls using oscillatory globe mill was performed for 7 minutes at the frequency of 15 $m.s^{-1}$. Disrupted algal material was used for analyses.

*Enzymatic disruption of algal cell walls*: Enzymatic disruption of cell walls was performed using filter bags F58 (ANKOM Technology, USA), Daisy incubator (ANKOM Technology, USA) and enzyme cellulase. Algal cell walls were enzymatic disrupted using cellulase (0.028 g of enzyme for 1 g of algal sample) for 24 hours at 40 °C. Algal samples (0.25 g) were weighed into filter bags, that were sealed and subsequently were inserted into incubation vessel containing 1700 ml acetate buffer (pH 4.6) tempered to 40 °C with adequate amount of cellulase. For correction was used filter bags without sample of alga. After incubation for 24 hours at 40 °C, filter bags were washed by distilled water. Thus enzymatic disrupted algal biomass was served to *in vitro* digestibility determination.

**Determination of *in vitro* digestibility:** Digestibility of dried matter (DMD) and organic matter (OMD) was performed according to method **(Mišurcová *et al*., 2010).** It was determined by the enzymatic-gravimetric *in vitro* method using Daisy incubator and filter bags F58. Algal biomass of each alga (0.25 g) was weighed into filter bags F58 that were sealed and further were inserted into incubation vessel. For correction, empty filter bag without sample of alga was simultaneously put into each incubation vessel. Digestibility was performed under different conditions: after 24 h hydrolysis by pepsin (3 g of enzyme for 6 g of algal biomass), after 24 h hydrolysis by pancreatin (3 g of enzyme for 6 g of algal biomass) and finally after combined hydrolysis using pepsin and pancreatin.

*Pepsin hydrolysis*: Pepsin hydrolysis was conducted in 1700 ml 0.1M HCl tempered to 40 °C with adequate amount of pepsin (Merck KGaA, Germany). Incubation vessel was inserted into the Daisy incubator for 24 hours at 40 °C. After the incubation time, filter bags were washed by distilled water, subsequently they were dried for 24 hours at 103±2 °C and finally they were burned in muffle furnace for 5 hours at 550 °C.

*Pancreatin hydrolysis*: The enzymatic hydrolysis was provided in 1700 ml phosphate buffer (pH 7.45) tempered to 40 °C that contained adequate amount of pancreatin (Merck KGaA, Germany). Further procedures were identical as in pepsin hydrolysis.

*Combined hydrolysis using pepsin and pancreatin*: Under combined hydrolysis the first pepsin hydrolysis was performed, consecutively filter bags were washed by distilled water and finally, pancreratic hydrolysis was conducted. Further procedures with filter bags were identical as in enzyme hydrolyses mentioned above.

*Calculation of digestibility*: Digestibility of the dried matter (DMD) and organic matter (OMD) were calculated according to equations (a)-(d).

(a) $DMD = 100 - \frac{100 \cdot DMR}{m_2 \cdot DM} \quad [\%]$ (b) $OMD = 100 - \frac{100 \cdot (DMR - AR)}{m_2 \cdot DM \cdot OM} \quad [\%]$

(c) $DMR = m_3 - m_1 \cdot c_1 \quad [g]$ (d) $AR = m_4 - m_1 \cdot c_2 \quad [g]$

where *DMD*-digestibility of the dried matter (%); OMD-digestibility of the organic matter (%); *DMR*-weight of algal sample after enzymatic hydrolysis and drying (g); DM-dry weight of algal sample (g/g); AR-weight of algal sample after enzymatic hydrolysis, drying and burning (g); *OM*-content of organic matter in algal sample (g/g); $m_1$-weight of empty filter bag (g); $m_2$-weight of sample (g); $m_3$-weight of filter bag containing sample after enzymatic hydrolysis and drying (g); $m_4$-weight of filter bag with algal sample after enzymatic hydrolysis, drying and burning (g); $c_1$ and $c_2$ are correction factors of weight of empty filter bags, $c_1$- $m_x/m_1$ ($m_x$ is weight of empty filter bag after enzymatic hydrolysis and drying) and $c_2$- $m_y/m_1$ ($m_y$ is weight of empty filter bag after enzymatic hydrolysis, drying and burning).

## RESULTS AND DISCUSSION

From data showen in Table 1, it is evident that the lowest effect on values of digestibility was observed with using pepsin, higher values were determined after pancreatin hydrolysis and finally, the highest hydrolysis effect was assessed after combined hydrolysis. It was observed that values of OMD in all investigated algae are higher, in comparison with values of DMD. This situation is supported by the fact, that dried matter contains indigestible compounds. Cyanobacterium *Sp. platensis* is greatly digestible and confirmed the hypothesis, that mechanical and enzymatic disruption methods or their combination stimulate only slightly positive rise or not at all its values of DMD and OMD.

*Sc. quadricauda* was evaluated as a poorest digestible green alga throughout all of type hydrolysis, *Chlorella. kessleri* and *Chlorella.* sp. were evaluated as more digestible algae (see Tab. 1).

**Tab. 1 DMD (%) and OMD (%) values of the investigated algae samples**

| | **PE** | | **PA** | | **PE + PA** | |
|---|---|---|---|---|---|---|
| | DMD [%] | OMD [%] | DMD [%] | OMD [%] | DMD [%] | OMD [%] |
| *Sc. quadricauda* | 25,9 | 33,2 | 30,0 | 38,4 | 37,2 | 44,9 |
| *Sc. quadricauda* (M) | 41,8 | 47,9 | 70,6 | 73,7 | 75,6 | 79,1 |
| *Ch. kessleri* | 53,2 | 59,9 | 71,3 | 75,7 | 80,3 | 83,4 |
| *Ch. kessleri* (M) | 61,1 | 66,6 | 75,3 | 79,4 | 83,8 | 86,6 |
| *Chlorella* sp. | 51,7 | 57,4 | 67,3 | 72,7 | 89,3 | 91,1 |
| *Chlorella* sp. (M) | 76,9 | 79,8 | 96,0 | 97,4 | 97,0 | 97,6 |
| *Sp. platensis* | 80,3 | 85,6 | 94,0 | 96,5 | 94,1 | 96,1 |
| *Sp. platensis* (M) | 80,0 | 85,3 | 93,7 | 95,4 | 93,4 | 95,3 |
| | **CE+ PE** | | **CE + PA** | | **CE + PE + PA** | |
| | DMD [%] | OMD [%] | DMD [%] | OMD [%] | DMD [%] | OMD [%] |
| *Sc. quadricauda* | 27,1 | 34,5 | 33,0 | 41,3 | 41,2 | 48,4 |
| *Sc. quadricauda* (M) | 63,3 | 66,8 | 79,3 | 82,5 | 82,4 | 84,4 |
| *Ch. kessleri* | 70,9 | 74,9 | 81,0 | 84,5 | 84,9 | 87,4 |
| *Ch. kessleri* (M) | 74,3 | 77,9 | 80,7 | 84,2 | 84,4 | 87,1 |
| *Chlorella* sp. | 91,7 | 92,7 | 95,4 | 96,9 | 97,3 | 98,3 |
| *Chlorella* sp. (M) | 96,1 | 96,6 | 98,1 | 98,9 | 98,0 | 99,1 |
| *Sp. platensis* | 79,3 | 84,9 | 93,5 | 96,0 | 93,7 | 95,8 |
| *Sp. platensis* (M) | 84,4 | 88,6 | 93,5 | 95,6 | 97,7 | 98,5 |

Under pepsin, pancreatin and combined hydrolysis, enzymatic disruption method significantly increase values of DMD and OMD in *Chlorella kessleri* and *Chlorella.* sp., unlike in *Sc. quadricauda*. In opposite, mechanical disruption of *Sc. quadricauda* caused significantly enhancement of its values of DMD and OMD, in other algal samples this proces reported less effectiveness in comparison with enzymatic method. The highest growth on values of digestibility was achieved when expose mechanical and enzymatic disruption methods together under all types of performed hydrolysis, further with combined hydrolysis was observed, that separate effect of mechanical and enzymatical disruption method in *Chlorella. kessleri* and *Chlorella* sp. caused positive effect on the increase of digestibility values at the same level, what is more application of these methods together indicated similar influence on increase of DMD and OMD.

## CONCLUSION

Enzymatic and mechanical disruption method positively influenced values of digestibility of investigated green freshwater algae. The highest efficiency on digestibility values was observed when these disruption methods effected together. This determination method of DMD and OMD appears as a suitable for rapid assessment of the effectiveness of disruption methods and estimate of the utilization of nutrients.

### REFERENCES

BECKER, E. W. 2007. Micro-algae as a source of protein. In *Biotechnology Advances*, vol. 25, p. 207-210.

CIFERI, O. 1983. *Spirulina*, the edible microorganism. In *Microbiological Reviews*, vol. 47, p. 551-578.

MIŠURCOVÁ, L. 2012. Seaweed Digestibility and Methods Used for Digestibility Determination. In Kim, S. K. *Handbook of Marine Macroalgae, Biotechnology and Applied Phycology,* Wiley-Blackwell, p. 285-301. ISBN 978-0-470-97918-1.

MIŠURCOVÁ, L., KRÁČMAR, S., KLEJDUS, B., VACEK, J. 2010. Nitrogen content, dietary fiber, and digestibility in algal food products. In *Czech Journal of Food Sciences*, vol. 28, p. 27-35.

SPOLAORE, P., JOANNIS-CASSAN, C., DURAN, E., ISAMBERT, A. 2006. Commercial application of microalgae. In *Journal of Bioscience and Bioengineering*, vol. 101, p. 87-96.

ZHOU, Z. P., LIU, L. N., CHEN, X. L., WANG, J. X., CHEN, M., ZHANG, Y. Z., ZHOU, B. C. 2005. Factors that effect antioxidant activity of c-phycocyanins from *Spirulina* platensis. In *Journal of Food Biochemistry*, vol. 29, p. 313-322.

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