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INFLUENCE OF PLANT-BASED SUBSTRATES ON CELL MASS PRODUCTION AND FERMENTATION ACTIVITY OF YEAST STRAINS

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1 INTRODUCTION AND AIMS

In 1950, 2.5 billion people lived on planet Earth, while the number of population will have reached 9 billion by 2050. Compared to the world population of 6.9 billion in 2010, it is expected to grow by over 2 billion within 40 years. Along with it the global need for food will increase by 70–90%, which means serious problems, tasks to be solved related mainly to calorie intake and meat consumption (Ministry of Agriculture, 2016). In connection with the abovementioned, satisfying vitamin and mineral needs of our farm livestock has to be focused on more and more because adequate quality forage will have greater importance in terms of providing the increasing demand for food. Single-cell proteins (SCP) produced by yeast (*Saccharomyces* spp.) or microalgae can play a significant role in this field.

The author's primary purpose was to optimize the SCP production process using *Saccharomyces (S.) cerevisiae* NCAIM Y.00200 and *Kluyveromyces (K.) marxianus* DSM 4908 yeast strains under laboratory conditions. This optimized process was later considered as control. Further aim was to increase SCP yield by vitamin supplementation in comparison to the optimized (control) processes. During the trials, yeast cell count, wet cell mass, dry matter, yeast protein, total sugar content of fermentation medium, specific growth rate, generation times, and protein yield values were determined and compared.

2 MATERIALS AND METHODS

All the experiments were carried out in the Department of Food Science (Faculty of Agricultural and Food Sciences) at Széchenyi István University. To start with, the fermentation parameters of two prechosen yeast strains (*S. cerevisiae* NCAIM Y. 00200 and *K. marxianus* DSM 4908) were optimized on molasses and corn steep liquor fermentation media, which were used as control for subsequent trials using vitamin supplementation.

The applied yeast strains were purchased in a freeze-dried and vacuum-packaged form. First the lyophilized preparation was rehydrated in saline (6.5 g/dm^3 NaCl), and then it was inoculated into

YGC broth, and was incubated at a temperature of 25°C for 48 hours. After that it was streaked onto YGC plates from broths to prepare pure culture. Before starting the experiments, the author prepared fresh cultures, and also carried out microscopical examination of the dyed preparations. After the microscopical examination of the cultures, inoculum was made by washing one lamella of yeast cell into 50 cm^3 saline solution (6.5 g/dm^3 NaCl), and then the required degree of cell concentration (10^6 cell/cm^3; Albertin et al. 2011) was set with a Buerker chamber. According to some literature sources, molasses was used as an 11–12% (Kutasi, 2007) or 18% solution (El-Gendy et al., 2013) to be fermented, while during final trials triple molasses and corn steep liquor dilution was applied. The used solutions, therefore, contained 655 cm^3 of effective plant-based fermentation medium and 1345 cm^3 of sterile saline solution (6.5 g/dm^3 NaCl). Prior to each research the reactor of the fermenter was sterilized at a temperature of 121ºC for 15 minutes in closed state to avoid post-infection. After calibration of the measuring instruments, 2 dm^3 of fermentation media were filled into the reactor under sterile conditions. At the beginning of the trials, based on literature sources, the required agitation, airflow rate, pH value and temperature were set, and the dissolved oxygen concentration was measured. After reaching the set parameters, the inoculum was injected into the raw material solution. The fermentation process was carried out for 72 hours, and several samples were taken during trials. In each case, three parallel measurements were carried out. The optimized process was later considered as control. On repeating the optimized processes, 1 cm^3/dm^3 of vitamin solution according to Wickerham was added to the raw material to be fermented. During the trials, yeast cell count, wet cell mass, dry matter, yeast protein, and total sugar content were determined and from these results specific growth rates, generation times and protein yield values were calculated.

3 RESULTS

3.1 Changes in dry matter content, wet cell mass, and yeast cell counts during optimized (control) and vitamin supplemented fermentation trials

Dry matter content, wet cell mass, and yeast cell counts were determined from the samples taken during optimized (control) fermentation processes, and then the experiments were repeated with vitamin supplementation using unchanged optimized (control) parameters. The yeast cell counts observed in optimized (control) and vitamin supplementation trials were almost identical (i.e., 8.4 to 8.5 $\log_{10}$ cfu/cm^3). The changes in dry matter content and wet cell mass are shown in Tables 1 and 2.

3.2 Changes in total sugar content of fermentation media, and in protein content of yeast during optimized (control) and vitamin supplemented fermentation trials

The total sugar content of fermentation media, and the protein content of yeasts were measured at h 0, 24, and 48 from the samples taken during optimized (control) and vitamin supplemented fermentation processes. The results can be seen in Tables 3 and 4.

Table 1: Dry matter values (g/100 g)☼ of the final products determined in the fermentation processes with and without vitamin supplementation

Time (hour)	A		B		C		D	
	experimental setting*							
	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation
0	6.18 ± 0.04^{b}	6.53 ± 0.01^{a}	2.91 ± 0.01^{a}	1.00 ± 0.5^{b}	3.12 ± 0.02^{b}	10.18 ± 0.02^{a}	5.00 ± 0.01^{b}	7.96 ± 0.01^{a}
24	8.28 ± 0.02^{a}	8.18 ± 0.02^{b}	3.60 ± 0.02^{a}	1.40 ± 0.35^{b}	4.32 ± 0.08^{b}	11.46 ± 0.01^{a}	8.50 ± 0.02^{a}	8.44 ± 0.01^{b}
48	12.15 ± 0.05^{b}	17.94 ± 0.05^{a}	5.51 ± 0.02^{a}	4.80 ± 0.10^{b}	6.80 ± 0.05^{b}	22.80 ± 0.15^{a}	10.50 ± 0.04^{b}	21.17 ± 0.01^{a}
72	13.62 ± 0.03^{b}	19.55 ± 0.02^{a}	8.66 ± 0.01^{a}	5.70 ± 0.20^{b}	9.16 ± 0.01^{b}	28.16 ± 0.07^{a}	13.47 ± 0.02^{b}	24.19 ± 0.01^{a}

☼ Values are means ± standard deviation based on three observations

[a,b] Subcolumn means within row and experimental setting without a common lowercase superscript differ ($P < 0.05$)

***** *Saccharomyces cerevisiae* NCAIM Y.00200 in molasses (A), and corn steep liquor (B) media, and *Kluyveromyces marxianus* DSM 4908 in molasses (C), and corn steep liquor (D) media

Table 2: Wet cell mass values (g/12 cm^3)☼ of the final product determined in the fermentation processes with and without vitamin supplementation

Time (hour)	A		B		C		D	
	experimental setting*							
	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation
0	0.166 ± 0.001^{b}	0.302 ± 0.003^{a}	0.145 ± 0.005^{a}	0.132 ± 0.002^{b}	0.161 ± 0.001^{a}	0.105 ± 0.003^{b}	0.123 ± 0.003^{a}	0.125 ± 0.004^{a}
24	0.231 ± 0.002^{b}	0.423 ± 0.003^{a}	0.236 ± 0.002^{a}	0.153 ± 0.001^{b}	0.217 ± 0.002^{a}	0.128 ± 0.003^{b}	0.150 ± 0.003^{a}	0.153 ± 0.001^{a}
48	0.340 ± 0.010^{b}	0.727 ± 0.002^{a}	0.321 ± 0.003^{a}	0.248 ± 0.002^{b}	0.330 ± 0.018^{a}	0.237 ± 0.002^{b}	0.270 ± 0.004^{a}	0.250 ± 0.002^{b}
72	0.490 ± 0.032^{b}	0.932 ± 0.030^{a}	0.420 ± 0.020^{a}	0.381 ± 0.004^{b}	0.462 ± 0.008^{a}	0.328 ± 0.006^{b}	0.579 ± 0.017^{a}	0.371 ± 0.003^{b}

☼ Values are means ± standard deviation based on three observations

[a,b] Subcolumn means within row and experimental setting without a common lowercase superscript differ ($P < 0.05$)

* *Saccharomyces cerevisiae* NCAIM Y.00200 in molasses (A), and corn steep liquor (B) media, and *Kluyveromyces marxianus* DSM 4908 in molasses (C), and corn steep liquor (D) media

Table 3: Changes in total sugar content (g/100 g)☼ of fermentation media during fermentation processes with and without vitamin supplementation

Time (hour)	A		B		C		D	
	experimental setting*							
	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation	Optimized (Control)	Vitamin supplementation
0	53.4±0.2^{a}	53.4±0.2^{a}	24.3±0.5^{a}	24.3±0.5^{a}	53.4±0.2^{a}	53.4±0.2^{a}	24.3±0.5^{a}	24.3±0.5^{a}
24	33.5±0.3^{a}	32.6±0.2^{b}	10.6±0.3^{a}	10.6±0.3^{a}	32.6±0.2^{b}	26.4±0.1^{b}	10.5±0.1^{a}	10.5±0.1^{a}
48	10.5±0.3^{a}	8.5±0.1^{b}	5.6±0.3^{a}	5.9±0.5^{a}	8.5±0.1^{b}	9.6±0.3^{b}	6.4±0.2^{a}	4.9±0.4^{b}
72	0.6±0.2^{b}	1.9±0.4^{a}	0.6±0.3^{a}	0.2±0.1^{a}	1.9±0.4^{a}	0.8±0.7^{a}	0.3±0.3^{a}	1.0±0.5^{a}

☼ Values are means ± standard deviation based on three observations

^{a,b} Subcolumn means within row and experimental setting without a common lowercase superscript differ ($P < 0.05$)

* *Saccharomyces cerevisiae* NCAIM Y.00200 in molasses (A), and corn steep liquor (B) media, and *Kluyveromyces marxianus* DSM 4908 in molasses (C), and corn steep liquor (D) media

Table 4: Changes in protein content of yeast (g/100 g)☼ during fermentation processes with and without vitamin supplementation

Time (hour)	A		B		C		D	
	experimental setting*							
	Control	Vitamin supplementation	Control	Vitamin supplementation	Control	Vitamin supplementation	Control	Vitamin supplementation
0	4.5±0.4[a]	4.5±0.4[a]	4.5±0.4[a]	4.5±0.4[a]	4.5±0.4[a]	4.5±0.4[a]	4.5±0.4[a]	4.5±0.4[a]
24	8.6±0.2[a]	8.9±0.6[a]	9.2±0.3[a]	9.1±0.5[a]	9.5±0.2[a]	9.6±0.2[a]	9.3±0.1[a]	8.9±0.4[a]
48	16.5±0.2[a]	17.0±0.2[a]	16.0±0.2[a]	16.2±0.4[a]	17.5±0.6[a]	16.2±0.4[b]	17.1±0.3[a]	16.0±0.2[b]
72	30.5±0.3[a]	28.0±0.5[b]	20.5±0.2[a]	20.2±0.1[a]	30.5±0.1[a]	30.1±0.1[b]	20.2±0.3[a]	20.0±0.5[a]

☼ Values are means ± standard deviation based on three observations

[a,b] Subcolumn means within row and experimental setting without a common lowercase superscript differ ($P < 0.05$)

* *Saccharomyces cerevisiae* NCAIM Y.00200 in molasses (A), and corn steep liquor (B) media, and *Kluyveromyces marxianus* DSM 4908 in molasses (C), and corn steep liquor (D) media

3.3 Changes in specific growth rates, generation times, and protein yields during optimized (control) and vitamin supplemented fermentation processes

Vitamin supplementation increased ($P < 0.05$) specific growth rates and, conversely, decreased ($P < 0.05$) generation times in each case. The most pronounced change was observed with *K. marxianus* DSM 4908 in corn steep liquor. During this experimental setting, with vitamin supplementation, the specific growth rate and generation time of *K. marxianus* DSM 4908 was 0.226 h^{-1} and 3.06 h, respectively. However, vitamin supplementation did not increase protein yield ($P > 0.05$).

4 CONCLUSIONS AND SUGGESTIONS

Vitamin supplementation mostly stimulated the dry matter production of yeast strains significantly, especially that of *K. marxianus* DSM 4908. It also increased ($P < 0.05$) specific growth rates (μ) and, conversely, decreased ($P < 0.05$) generation times in each case. The highest μ value (0.226 h^{-1}) and the shortest generation time (3.06 h) were measured for *K. marxianus* in corn steep liquor with vitamin supplementation and, because the corresponding dry matter values were also beneficial, this combination of yeast strain and fermentation medium is suggested for further use. The sugar contents of fermentation media measured in h 72 of the fermentation trials were 0.3–1.9 g/100 g in control samples, and with vitamin supplementation they were 0.2–1.9 g/100 g. The protein contents of yeasts measured in controls at the end of the 3-day processes were 20.2–30.5 g/100 g, and with vitamin supplementation they were 20.0–30.1 g/100 g. Vitamin supplementation, therefore, did not increase ($P > 0.05$) the protein content of yeasts or their protein yield (0.5–0.7 g/g), nevertheless the produced SCP, described in this dissertation, can be used as a feedstuff additive to satisfy the protein needs of livestock. Thanks to advanced technologies, it is more and more frequent that final products accrued from recycling food by-products are made suitable not only for feeding ruminants. Accordingly, the SCP produced in the author's trials may have a wide use following disruption of yeast cell walls.

5 NEW SCIENTIFIC FINDINGS

1. Single-cell protein (SCP) production has been optimized in a diluted molasses fermentation medium using *Saccharomyces cerevisiae*. Under optimized environmental conditions (temperature: 30°C, pH value: 5.5, agitation speed: 200 rpm, airflow rate: 1.5 vvm) in a fermentation medium containing 17.8 g/100 g sugar on average, *S. cerevisiae* NCAIM Y.00200 produced a cell mass with 10.16 g/100 g of protein content, resulting in 0.5 g/g of protein yield within 72 hours.

2. SCP production has been optimized in a diluted corn steep liquor fermentation medium applying *S. cerevisiae*. Under optimized environmental conditions (temperature: 30°C, pH value: 5.5, agitation speed: 400 rpm, airflow rate: 1.5 vvm) in a fermentation medium containing 8.1 g/100 g sugar on average, *S. cerevisiae* NCAIM Y.00200 produced a cell mass with 6.83 g/100 g of protein content, resulting in 0.7 g/g of protein yield within 72 hours.

3. SCP production has been optimized in a diluted molasses fermentation medium applying *Kluyveromyces marxianus*. Under optimized environmental conditions (temperature: 30°C, pH value: 4.5, agitation speed: 300 rpm, airflow rate: 1.5 vvm) in a fermentation medium containing 17.8 g/100 g sugar on average, *K. marxianus* DSM 4908 produced a cell mass with 10.16 g/100 g of protein content, resulting in 0.5 g/g of protein yield within 72 hours.

4. SCP production has been optimized in a diluted corn steep liquor fermentation medium applying *K. marxianus*. Under optimized environmental conditions (temperature: 30°C, pH value: 4.5, agitation speed: 300 rpm, airflow rate: 1.5 vvm) in a fermentation medium containing 8.1 g/100 g sugar on average, *K. marxianus* DSM 4908 produced a cell mass with 6.73 g/100 g of protein content, resulting in 0.6 g/g of protein yield within 72 hours.

5. The specific growth rates of *S. cerevisiae* NCAIM Y.00200 and *K. marxianus* DSM 4908 can be increased ($P < 0.05$) by

supplementation at 1 cm^3/dm^3 with a vitamin solution according to Wickerham and, conversely, the generation times of these strains can be decreased ($P < 0.05$) during their growth in diluted molasses and corn steep liquor fermentation media.

6 REFERENCES

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7 SCIENTIFIC PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

Peer Reviewed Papers

In English

1. **Molnár, J.**, Ásványi, B. (2019) Studying growth characteristics of yeast strains on vegetal fermentation media and with vitamin supplementation. *Acta Alimentaria* 48 (2), 143-149. [**JIF: 0,384**]
2. **Molnár, J.**, Vasas, D., Ásványi, B. (2018) Incorporating single cell proteins in the diet of IBD patients. *Journal of Food Investigation* 64 (4), 2294-2296.
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1. **Molnár, J.,** Lakatos, E., Ásványi, B. (2018) Influencing the growth kinetics of yeast strains by vitamin supplementation. *Acta Agraria Debreceniensis–Agrártudományi Közlemények* 74, 113-115.

In Hungarian

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