Para-rubber Seed Kernel Fermentation Using *Aspergillus oryzae* and *Saccharomyces cerevisiae*: Targeting for Aquafeed

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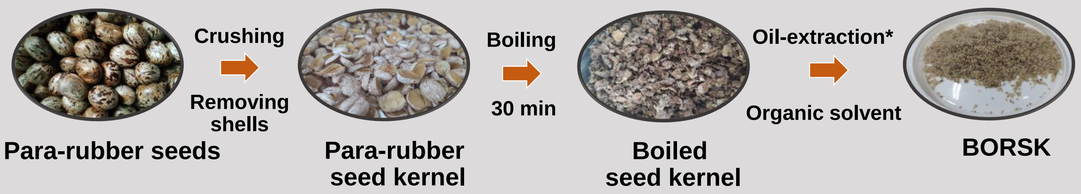
Introduction

Para-rubber is an important economic crop in Thailand, having a cultivation area of up to 5.36 million acres and yielding an abundance of rubber seeds. However, only a small portion of the para-rubber seed kernels (PRSK) is utilized, and the remainder is left to rot. The PRSK can be used as an ingredient in animal feed if appropriate processing methods are applied. Fermentation can be a suitable technique to enhance the nutritional value of PRSK. Therefore, this study aimed at increasing the nutritional value of PRSK employing two fermentation steps using *A. oryzae* and *S. cerevisiae*.

Materials and Methods

1) Kernel preparation for fermentation

- **Boiled and oil-extracted para-rubber seed kernel (BORSK) preparation**



*Boiled seed kernel was soaked overnight in 95 % ethanol at the ratio of 1 : 6 (solid to solvent) at ambient temperature. The whole mixture was heated at 80 ˚C and agitated for 8 h on a hot plate with magnetic stirrer. Then, the solid was allowed to settle for 12 h, separated and oven dried at 60˚C for 24 h.

2) Two-step fermentation

Step 1 : Fermentation of BORSK with *A. oryzae* (BORSKFA)

The fermentation was at ambient temperature for 192 h. The proximate composition of the samples was examined to select the BORSKFA product for subsequent fermentation procedures.

*Distilled water

Step 2 : BORSKFA fermentation with *S. cerevisiae* and optimization of factors (sugar, DW and time).

- ***S. cerevisiae* level optimization for BORSKFA fermentation (results shown in Table 2)**

BORSKFA (30 g) + *S. cerevisiae* levels 0, 0.2, 0.3, 0.4 and 0.5% + 0.2% Sugar, 50% DW for 48 h

- **Sugar level optimization for BORSKFA fermentation (results shown in Table 3)**

BORSKFA (30 g) + 5% *S. cerevisiae* + Sugar levels 0, 0.2, 0.3, 0.4 and 0.5% + 50% DW for 48 h

- **Distilled water level optimization for BORSKFA fermentation (results shown in Table 4)**

BORSKFA (30 g) + 5% *S. cerevisiae*, 0.2% Sugar + DW levels 0, 50, 66.67, 83.33 and 100% (w/v) + 48 h

- **Suitable BORSKFA fermentation time (results shown in Table 5)**

BORSKFA (30 g) + 5% *S. cerevisiae* , 0.2% Sugar and 50% DW (w/v) + Time 0, 24, 48 and 72 h

Results

Table 1. Proximate composition (% dry matter) of BORSK fermented with 0, 1 and 2% *A. oryzae* and 20, 40, 60 and 80% DW (w/v), respectively, for 192 h.

Treatments (% of *A. oryzae* + % of DW)	% dry matter		
	Protein	Lipid	Ash
Original sample (BORSK)	28.89 ± 0.03	18.23 ± 0.22	3.41 ± 0.45
T1 (0% + 20%)	26.55 ± 0.22^{a}	34.10 ± 0.40^{c}	3.66 ± 0.03^{ab}
T2 (0% + 40%)	26.24 ± 0.46^{a}	34.72 ± 1.19^{c}	3.62 ± 0.06^{ab}
T3 (0% + 60%)	31.20 ± 0.67^{b}	41.43 ± 1.59^{cd}	5.09 ± 0.09^{bcd}
T4 (0% + 80%)	34.26 ± 0.11^{b}	48.58 ± 0.00^{d}	5.80 ± 0.16^{cde}
T5 (1% + 20%)	32.71 ± 0.60^{b}	17.24 ± 0.45^{b}	3.11 ± 0.53^{a}
T6 (1% + 40%)*	42.88 ± 0.47^{c}	3.36 ± 0.01^{a}	5.12 ± 0.81^{bcd}
T7 (1% + 60%)	47.10 ± 0.18^{d}	9.71 ± 6.12^{ab}	7.51 ± 0.18^{e}
T8 (1% + 80%)	47.45 ± 0.62^{d}	35.42 ± 0.15^{c}	6.84 ± 0.57^{de}
T9 (2% + 20%)	32.93 ± 0.52^{b}	15.81 ± 0.85^{ab}	3.05 ± 0.71^{a}
T10 (2% + 40%)	42.76 ± 0.70^{c}	3.76 ± 1.77^{a}	4.47 ± 0.63^{abc}
T11 (2% + 60%)	44.68 ± 2.93^{cd}	12.32 ± 9.05^{ab}	6.35 ± 0.58^{de}
T12 (2% + 80%)	45.96 ± 0.20^{cd}	34.14 ± 0.10^{c}	6.54 ± 0.01^{de}
p-value	0.000	0.000	0.000

Values are mean ± SD, n=2. Means of main effects in same column with different superscripts are significantly different ($p < 0.05$).
*This treatment was selected for further fermentation with *S. cerevisiae.*

Table 2. Proximate composition (% dry matter) of BORSKFA[1] fermented with 0, 2, 3 , 4, and 5% *S. cerevisiae* for 48 h.

Treatment (BORSKFA + % of *S. cerevisiae*)	% dry matter		
	Protein	Lipid	Ash
T1 (BORSKFA + 0%)	42.55 ± 0.27^{a}	20.19 ± 0.37^{b}	4.94 ± 0.10^{a}
T2 (BORSKFA + 2%)	45.09 ± 0.70^{b}	22.23 ± 0.30^{c}	5.29 ± 0.20^{b}
T3 (BORSKFA + 3%)	44.79 ± 0.44^{b}	20.86 ± 0.48^{b}	5.20 ± 0.03^{ab}
T4 (BORSKFA + 4%)	44.57± 0.39^{b}	20.42 ± 0.02^{b}	5.06 ± 0.08^{ab}
T5 (BORSKFA + 5%)*	44.52 ± 0.10^{b}	18.45 ± 0.23^{a}	5.11 ± 0.03^{ab}
p-value	0.000	0.000	0.021

Values are mean ± SD, n=3. Means of main effects in same column with different superscripts are significantly different ($p < 0.05$).
[1]Boiled and oil-extracted rubber seed kernel fermented with 1% *A. oryzae* and 40% DW.
*This treatment was selected for further fermentation to obtain the suitable sugar level.

Table 3. Proximate composition (% dry matter) of BORSKFA[1] fermented with 5% *S. cerevisiae* at 0, 0.2, 0.3, 0.4 and 0.5% sugar for 48 h.

Treatment (BORSKFA + % of sugar)	% dry matter		
	Protein	Lipid	Ash
T1 (BORSKFA + 0%)	45.83 ± 0.23^{a}	20.24 ± 0.15^{a}	5.19 ± 0.57^{a}
T2 (BORSKFA + 0.2%)	45.46 ± 0.57^{a}	20.43 ± 1.13^{a}	5.03 ± 0.50^{a}
T3 (BORSKFA + 0.3%)	45.94 ± 0.21^{ab}	20.54 ± 0.53^{a}	4.85 ± 0.21^{a}
T4 (BORSKFA + 0.4%)	45.94 ± 0.22^{ab}	20.57 ± 0.16^{a}	5.26 ± 0.23^{a}
T5 (BORSKFA + 0.5%)*	46.80 ± 0.32^{b}	20.93 ± 0.27^{a}	5.29 ± 0.31^{a}
p-value	0.008	0.691	0.058

Values are mean ± SD, n=3. Means of main effects in same column with different superscripts are significantly different ($p < 0.05$).
[1]Boiled and oil-extracted rubber seed kernel fermented with 1% *A. oryzae* and 40% DW.
*This treatment was selected for further fermentation to obtain a suitable DW level.

Table 4. Proximate composition (% dry matter) of BORSKFA[1] fermented with 5% *S. cerevisiae* and 0.5 sugar at 0, 50, 66.67, 83.33 and 100% DW (w/v) for 48 h.

Treatment (BORSKFA 5% + % of DW)	% dry matter		
	Protein	Lipid	Ash
T1 (BORSKFA + 0%)	37.19 ± 0.12^{a}	15.39 ± 0.28^{a}	4.57 ± 0.23^{a}
T2 (BORSKFA + 50%)	43.30 ± 0.79^{b}	19.31 ± 0.20^{b}	5.44 ± 0.13^{b}
T3 (BORSKFA + 66.67%)	44.63 ± 0.32^{c}	20.40 ± 0.23^{c}	6.14 ± 0.18^{c}
T4 (BORSKFA + 83.33%)	46.26 ± 0.32^{d}	21.75 ± 0.13^{d}	6.24 ± 0.07^{c}
T5 (BORSKFA + 100%)*	46.76 ± 0.64^{d}	20.88 ± 0.28^{c}	6.46 ± 0.10^{c}
p-value	0.000	0.000	0.000

Values are mean ± SD, n=3. Means of main effects in same column with different superscripts are significantly different ($p < 0.05$).
[1]Boiled and oil-extracted rubber seed kernel fermented with 1% *A. oryzae* and 40% DW.
*This treatment was selected for further fermentation to obtain a suitable fermentation time.

Table 5. Proximate composition (% dry matter) of BORSKFA[1] fermented with 5% *S. cerevisiae,* 0.5 sugar and 100% DW for 0, 24, 48 and 72 h.

Treatment (BORSKFA + fermentation time)	% dry matter		
	Protein	Lipid	Ash
T1 (BORSKFA + 0 h)	39.65 ± 0.25^{a}	19.12 ± 0.19^{a}	4.77 ± 0.12^{a}
T2 (BORSKFA + 24 h)	44.88 ± 0.42^{bc}	22.00 ± 0.60^{b}	5.50 ± 0.21^{b}
T3 (BORSKFA + 48 h)	44.34 ± 0.58^{b}	22.15 ± 0.33^{b}	5.74 ± 0.02^{b}
T4 (BORSKFA + 72 h)*	45.71 ± 0.14^{c}	22.78 ± 0.32^{b}	5.63 ± 0.26^{b}
p-value	0.000	0.000	0.001

Values are mean ± SD, n=3. Means of main effects in same column with different superscripts are significantly different ($p < 0.05$).
[1]Boiled and oil-extracted rubber seed kernel fermented with 1% *A. oryzae* and 40% DW.
*This treatment was considered a suitable time for fermentation.

Conclusion

The first step, BORSK which was fermented for 192 h with 1% *A. oryzae*, 0.75 % urea, and 40% DW, exhibited increased protein and yielded the best results, and was then used for subsequent fermentation.

The second step of fermentation with 5% *S. cerevisiae*, 0.5% sugar, 100% DW (w/v) for 72 h was the optimal combination of the factors, increasing protein level to 45.71 ± 0.14% (dry matter basis).

It can be concluded that this two-step processing method using *A. oryzae* and *S. cerevisiae* as reported above can be used to improve the nutritional value of PRSK.