Elevated vitamin B_{12} levels in lupin tempeh by *in situ* fortification

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Background

Tempeh is a traditional, fungal fermented Indonesian product, usually made from soybeans. It is known to contain vitamin B_{12}, essential for a healthy diet. Therefore, tempeh is of particular interest for vegan diets since B_{12} is normally found only in animal derived products.

Vitamin B_{12} in tempeh is associated with the presence of opportunistic pathogens *Klebsiella pneumoniae* and *Citrobacter freundii*.

Levels of B_{12} in tempeh are currently not sufficient to sustain the recommended daily intake. In addition, the use of food-grade bacteria instead of *K. pneumoniae* is preferred.

Objectives & approach

- This study aims to boost the amount of vitamin B_{12} in lupin tempeh by bacterial biosynthesis using food grade bacteria *Lactobacillus reuteri* and *Propionibacterium freudenreichii* in co-culture with the fungus *Rhizopus oryzae*.
- Lupin beans were tested as an alternative substrate for soybeans because of its similar protein content.
- Tempeh quality was evaluated for microbial composition, texture and volatile organic compounds (VOC's).

Results

Lupin tempeh was successfully produced using a co-culture of fungus *R. oryzae* and different bacteria, and visually appeared of good quality (Fig. 1) compared to tempeh fermented with fungus only.

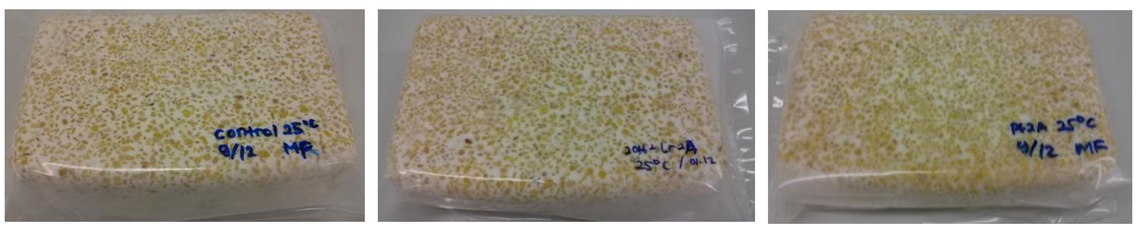

Figure 1. Lupin tempeh (left: control, middle: co-cultured with *L. reuteri*, right: co-cultured with *P. freudenreichii*)

In contrast to *P. freudenreichii*, growth of *L. reuteri* was not observed in the lupin tempeh. To boost vitamin B_{12} levels, different initial inocula of *P. freudenreichii* were used.

A significant increase (0.97 µg/100 g) of vitamin B_{12} was found when using an inoculum of 10^7 cells *P. freudenreichii* (Table 1).

Table 1**:** Vitamin B_{12} content and initial/final bacterial counts in lupin tempeh produced by only *R. oryzae* (Control) and co-inoculation with *Klebsiella pneumonia* (Kp), *Lactobacillus reuteri* (Lr) or different initial counts of *P. freudenreichii* (Pf 10^3,10^5 and 10^7). Tempeh was incubated for 2 days at 25 °C. Means with different superscripts are significantly different ($P<0.05$).

Processing scenario	Vitamin B_{12} (µg/100 g)	Initial bacterial count (log CFU/g)	Final bacterial count (log CFU/g)
Control	0	-	-
Kp	**0.96** ± 0.06	2.6 ± 0.1	9.5 ± 0.4
Lr	0.01 ± 0.01	2.62 ± 0.04	-
Pf 10^3	0.01 ± 0	3 ± 0.3	5 ± 0
Pf 10^5	0.05 ± 0.02^a	5.1 ± 0.2	7.8 ± 1.7
Pf 10^7	**0.97** ± 0.07^b	6.7 ± 0.2	8.4 ± 0.1

VOC's, determined by GCMS, showed no major differences between processing scenario's (Fig. 2).

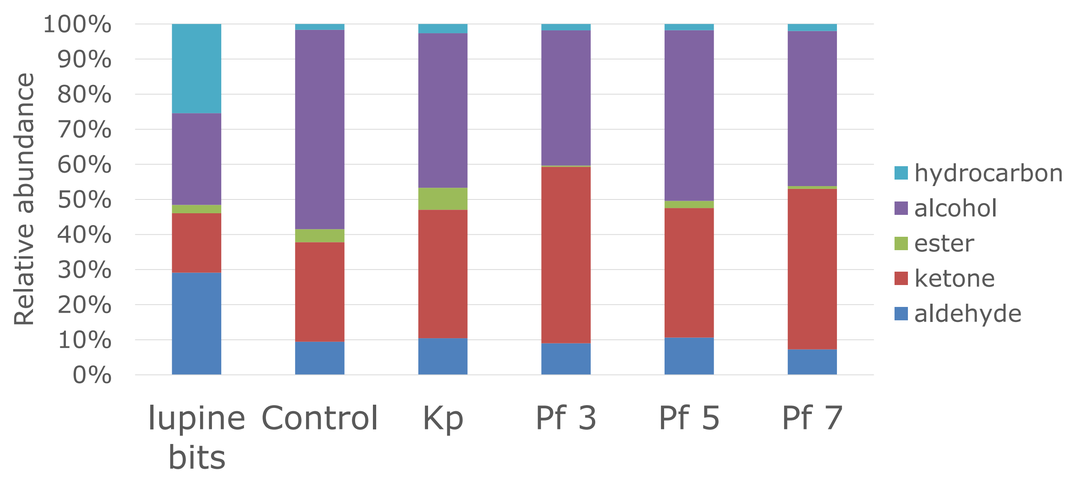

Figure 2. Relative abundance of chemical classes of VOC's present in raw lupine bits and lupin tempeh produced by different process scenarios (*R. oryzae* (Control) and co-inoculation with *K. pneumoniae* (Kp) or different initial counts of *P. freudenreichii* (Pf 10^3,10^5 and 10^7, indicated as Pf 3, Pf 5 and Pf 7 respectively). All tempeh's were incubated for 2 days at 25 °C.

The main compounds found were 2-butanone, ethanol, 3-methyl-1-butanol, 2-butanol, acetone and acetaldehyde.

Other parameters, such as microbial composition and texture, were not affected by the use of a bacterial co-inoculation.

Conclusions

- Co-inoculation of tempeh with *R. oryzae* and *P. freudenreichii* leads to a significant increase in vitamin B_{12}.
- *In situ* fortified lupin tempeh is a sustainable vegetable source of protein and vitamin B_{12}.

Acknowledgement

We would like to thank Prof. Dr Ir Antonius Suwanto from Bogor Agricultural University (Indonesia) for providing the bacterial isolate of *Klebsiella pneumoniae*.

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