

## Abstract

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# Exploring sustainable protein sources: Enhancing the nutritional profile of lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*) seeds via fermentation

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Food security constitutes a worldwide concern closely correlated with population growth. By 2050, the global population is expected to reach 9.3 billion<sup>(1)</sup>. The rising population, along with increasing life expectancy and shifts toward Western dietary patterns, is expected to drive higher food demand and contribute to a rise in metabolic conditions<sup>(2)</sup>. In this context, looking for alternative and sustainable food and protein sources is imperative. Pasture legumes including lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*) are becoming popular as they can be used as an alternative protein and functional food source. Both crops play an important role in New Zealand's agriculture. Their seeds can be used in human nutrition as alternative food and protein options; however, the presence of anti-nutritional factors (ANF) and their distinct taste make them less favourable for human consumption. Fermentation can be used as a possible strategy to mitigate these limitations. *Lactobacillus* fermentation was conducted using *Lactococcus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus casei*. Proximate composition and mineral content were determined following Association of Official Analytical Chemists (AOAC) methods. Total phenol content (TPC), total flavonoid content (TFC) and antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) and ANF including phytic acid, trypsin, and chymotrypsin inhibition were assessed using colourimetric techniques. For the enzyme inhibition assays, enzyme-substrate reactions were performed with sample extracts before measurement. All the experiments were replicated three times, and the results were expressed as mean  $\pm$  SD. A factorial analysis of variance (ANOVA) was conducted (4 legume seed samples  $\times$  3 LAB cultures) with a Tukey's post-hoc test for mean comparison at  $P < 0.05$  using IBM SPSS Statistics 29.0. All the legume seeds demonstrated high nutritional content, with crude protein and fibre levels around 40 and 16% respectively. The seeds were also rich in minerals, particularly magnesium, phosphorus, iron and zinc. In addition, fermentation led to an increase ( $P < 0.05$ ) in TPC, TFC and antioxidant activity, while significantly reducing ANF. For instance, fermentation led to an increase in TPC (18.8 to 47.1% increase), TFC (9.6 to 34.5% increase) and AOA via DPPH and ABTS. *Lactobacillus* fermentation has proven to be an effective processing technique to enhance the nutritional value of lucerne and red clover seeds. These findings support the potential of using fermentation to develop novel and sustainable protein sources, contributing to improved dietary quality and nutrition. Moreover, further work to study the effect of fermentation on the nutrient digestibility of lucerne and red clover seeds is warranted.

**Keywords:** ANF; antinutritional factors, LAB; lactic acid bacteria, AOAC; association of official agricultural chemists

**Ethics Declaration:** Yes

## References

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