Isolasi protein ikat tiamin dengan Metode Salting Out pada kacang lima (Phaseolus lunatus) = Isolation of thiamine binding protein (TBP) from lima beans (Phaseolus lunatus) using Salting Out Method

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Abstrak

Latar Belakang
Tiamin merupakan mikronutrien yang dibutuhkan dalam proses metabolisme energi. Defisiensi tiamin dapat menyebabkan berbagai kelainan jangka pendek dan jangka panjang, terutama pada populasi khusus, seperti pasien dengan diabetes atau stunting. Meskipun pentingnya kecukupan tiamin, belum terdapat standar tiamin serum bagi populasi Indonesia. Hal ini disebabkan oleh metode pengujian yang belum optimal untuk dilakukan pada sampel besar. Karena itu dikembangkan metode pengukuran yang lebih ekonomis dan praktis menggunakan prinsip ELISA namun menggunakan protein ikat tiamin. Protein ini telah ditemukan dalam berbagai spesies kacang-kacangan. Penelitian ini mengeksplorasi keberadaannya pada spesies kacang yang masih kurang diteliti, yaitu kacang lima (Phaseolus lunatus)
Metode
Kacang lima dihaluskan dan dibuat tiga sampel kacang, masing-masing sebanyak 5 gram. Tiap sampel dilarutkan menggunakan dapar fosfat. Larutan tersebut disentrifugasi dan supernatan diambil. Dilakukan proses salting out dengan ammonium sulfat, lalu larutan kembali disentrifugasi. Presipitat diisolasi dan dilarutkan dengan dapar fosfat lalu menjalani proses dialisis. Kadar protein diukur menggunakan spektrofotometer. Ditambahkan larutan tiamin pada sampel hasil salting out, kemudian dilakukan dialisis kesetimbangan untuk mengukur aktivitas pengikatan tiamin. Absorbansi dialisat dibaca untuk menghitung jumlah tiamin terikat.
Hasil
Penelitian ini menunjukkan bahwa kacang lima mengandung rata-rata 7,61 gram protein/100 gram kacang. Ditemukan aktivitas pengikatan tiamin yang bervariasi antara 573,16 dan 878,09 g/10 gram kacang dengan rasio tiamin terikat per jumlah protein 0,95g/mg protein.
Kesimpulan
Ditemukan protein ikat tiamin pada kacang lima dengan aktivitas yang tinggi dibandingkan dengan sumber lainnya.
.....Introduction
Thiamine is a micronutrient essential for energy metabolism, and its deficiency may lead to various disorders, especially on certain populations, such as diabetic and stunting patients. In Indonesia, there is no established serum thiamine standard, partly due to the unoptimal testing methods available. Therefore, a more economical and practical measurement method was developed using the ELISA principle but using thiamine binding protein found in various species of beans. This research explores its presence in a lesser-studied lima bean (Phaseolus lunatus)
Method
Lima beans were ground and three samples are prepared, each containing 5 grams of bean. Each sample is dissolved using phosphate buffer. The solution was centrifuged and the supernatant was collected.

Ammonium sulfate was added to precipitate protein, then the solution was centrifuged again. The precipitate was isolated and dissolved in phosphate buffer, then dialysed. Protein levels were measured using a spectrophotometer. Thiamine solution was added to the salted out sample, then equilibrium dialysis was carried out to measure the thiamine binding activity. Absorbance reading of the dialysate were used to quantify bound thiamine.

Results

Lima beans contain an average of 7.61 grams of protein/100 grams. Thiamine binding activity ranged from 573.16 to 878.09 g per 10 grams of beans with a ratio of bound thiamine per amount of protein of 0.95 g/mg protein.

Conclusion

Thiamine binding protein was found in Lima beans with high activity compared to other sources.