

UV/VIS SPECTROPHOTOMETRY APPLIED TO DETERMINATE PHOSPHORUS IN ALMOND MILK

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REZUMAT. Spectrometria UV/VIS a fost utilizată pentru a determina conținutul de fosfor în laptele de migdale. Probele de lapte de migdale au fost supuse la calcinare, în scopul îndepărtării materialului organic. Fosfații au reacționat cu moliadatul rezultând un complex: $[(\text{MoO}_2 \cdot 4\text{MoO}_3)_2 \cdot \text{HPO}_4]$, care a fost redus cu un agent de reducere, cum ar fi acidul ascorbic, la albastru molibden. Două tipuri de probe de lapte de migdale (lapte de migdale normal și lapte de migdale cu Omega 3), prelevate de la Universitatea Insulele Baleare, Spania, Palma de Mallorca, au fost analizate. Conținutul de fosfor din probele de lapte de migdale a fost de aproximativ 11,069-11,97mg/100, iar din laptele de migdale cu Omega 3 a fost 13,029-13,673 mg/100g

Cuvinte cheie : lapte de migdale, fosfor, spectrofotometrie

ABSTRACT. UV/VIS spectrofotometry was used to determine phosphorus contents in almond milk. The samples of almond milk were subjected to calcination in order to remove organic material. The phosphates were reacting with molybdate resulting a complex: $[(\text{MoO}_2 \cdot 4\text{MoO}_3)_2 \cdot \text{HPO}_4]$, which was reduced with a reducing agent, like ascorbic acid at molybdenum blue. Two types of almond milk samples (normally almond milk and almond milk with omega 3), provided from University of Balearic Islands, Spain, Palma de Mallorca were analyzed. Phosphorus content from almond milk samples was about 11,069-11,97 mg/100 and from almond milk with omega 3 samples were 13,029 - 13,673 mg/100g.

Keywords: almond milk, phosphorus, spectrophotometry

1. INTRODUCTION

UV/VIS Spectrophotometry is used to determine the absorption on transmission of UV/VIS light (180 to 820 nm) by a sample. It can also be used to measure concentrations of absorbing materials based on developed calibration curves of the material. A sample is placed in the UV/VIS beam and a graph of the transmittance or absorbance versus the wavelength is obtained.

Alternatively, samples are prepared in known concentration and their absorbance is read by the UV/VIS Spectrophotometer. Results are then graphed to make a calibration curve from which the unknown concentration can be determined by its absorbance. This method was used in this research to determine phosphorus content from two types of almond milk samples: almond milk and almond milk with omega 3.

Almond milk is one of the latest products to be used as a substitute of animal milk. It is the perfect alternative for people suffering from lactose-intolerance (*Iaconoa G (2008)*) being easily digestive. Almond milk is rich in vitamin E, a known antioxidant, and thus is believed to good for those undergoing detoxification programs.

Almond milk contains high levels of unsaturated fat and has been associated with lower risk of heart attack (*Jenkins et al, 2002*). Has been found to contain no cholesterol (*Spiller et al, 1992*), making it extremely good for those suffering from high cholesterol problem.

2. METHOD AND MATERIALS

2.1. Equipment, materials and reagents

- analytical balance
- spectrophotometer UV-VIS Varian Carry 50
- calcinations furnace

- saggars
- water bath
- 50 ml calibrated flask
- 50 ml barrels
- 5, 10 ml graduated pipettes
- Glass dish (1 cm)
- Filter paper
- Distilled water
- hydrochloric acid
- 50% % potassium hydroxide solution (KOH): 50 g KOH were dissolved in 50 ml water
- molybdate-acid ascorbic solution, fresh- 25 ml sodium molybdate were mixed in a 100 ml calibrated flask with 10 ml acid ascorbic solution, 5% and brought to sign
- standard phosphate solution : a dilution of 1:100 from standard solution were made; this solution will have 0,01 mg P/ml
- zinc oxide
- phosphorus solution for calibration curve: 0, 5, 10, 20, 30, 40 µg/ml

2.2. Samples and phosphorus solution for calibration curve preparation

The samples of almond milk, respectively , almond milk with omega 3 were brought to 20-25° C and mixed well for fat homogenization. Briefly, 10g sample, weighed on analytical balance with accuracy of 0.1 mg, were mixed with 0,5 g zinc oxide and dried at 110 ° C for 1-2 h, then charred until stopped fume. The samples were calcined at 525 ° C during 4 hours from the time when the calcinations furnace was 525 ° C, until a white ash was obtained.

The saggars were then cooled at room temperature. When they were cold 5 ml water and 5 ml HCl were added and boiled for 5 minutes on a warming plate. They are filtered in a 50 ml calibrated flask , and washed the content of saggars with 5 ml hot water for 4-5 times.

The content of calibrated flask was then cooled at room temperature and neutralized by adding KOH , 50 % solution, until the solution was opaque(the precipitation of Zn(OH)₂ started). Then, HCl was added until the precipitate disappeared and 2 more drops. The solution was let at room temperature to cool and brought to sign at 50 ml with water. In parallel a procedural blank was prepared.

10 ml from prepared solution were transferred into a 50 ml calibrated flask, together with 20 ml of molybdate-acid ascorbic solution, and brought to sign with water.

Table 1 shows how phosphorus calibration curve solution were prepared.

2.3. Samples treatment and absorbance reading

The solutions prepared for calibration curve , together

with samples solution were placed in a metal basket after were closed with cap. The basket was then immersed in a boiling water bath for 15 minutes. After 15 minutes, the flasks were cooled under water jet until they have 20-30 °C. The colour of solution was blue, due to reaction of phosphorus with molybdate-acid ascorbic solution.

Table 1: Phosphorus calibration curves solution.

Solution	0	1	2	3	4	5
The volume of phosphorus solution, 0,01 mgP/ml	0	0,5	1,00	2,00	3,00	4,00
Water , ml	15	14,5	14	13	12	11
Molybdate-acid ascorbic solution , ml	20	20	20	20	20	20
Water dilution at:	50	50	50	50	50	50
Conc P in µg	0	5	10	20	30	40

The solutions absorbance were read at a wavelength of 823 ± 1 nm. , first calibration curve and then samples solution.

3. RESULTS AND DISCUSSION

The content of phosphorus from almond milk samples was expressed in g/100 g and was calculated with this formula:

$$P, \text{ g/100 g} = [(V_2/V_1) \cdot C_{\text{citita}} \cdot 50] / m \quad (1),$$

where

m = amount of samples weighted

V₁ = the volume of solution utilized for color reaction

V₂ = the volume where the ash was brought, 50 ml

C_{read} = the amount of phosphorus from calibration curve

From read absorbance , the calibration curve was build, by repreasenting the absorbance in relation with solution concentrations (fig.1.)

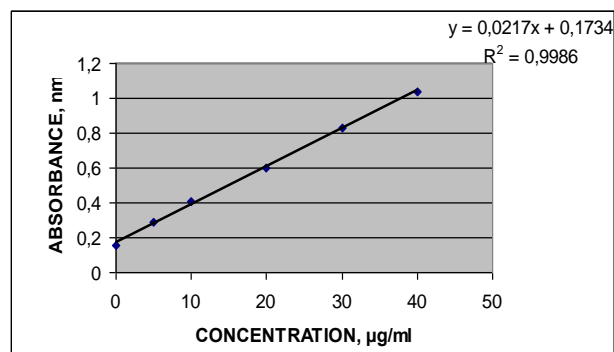


Fig.1 Calibration curve for phosphorus solutions.

Also, the method was designed to animal milk, the results showed that the method can be successfully applied on vegetal milk.

Table 2 shows the result obtained for almond milk, respectively, almond milk with omega 3.

Table 2: Phosphorus values from almond milk

Sample	Phosphorus concentration, µg/ml	Phosphorus content, mg/100 g
Almond milk 1	24,1459	11,970
Almond milk 2	22,3288	11,069
Almond milk 3	23,1256	11,464
Almond milk 4	24,1521	11,973
Almond milk 5	22,5672	11,187
Almond milk 6	24,1526	11,973
Almond milk 7	23,6793	11,739
Almond milk 8	22,4456	11,127
Almond milk 9	23,5148	11,657
Almond milk 10	24,0378	11,916
Almond milk with omega3-1	27,1577	13,530
Almond milk with omega3-2	26,8457	13,375
Almond milk with omega3-3	27,1256	13,514
Almond milk with omega3-4	26,1521	13,029
Almond milk with omega3-5	26,5672	13,236
Almond milk with omega3-6	27,1526	13,527
Almond milk with omega3-7	26,6793	13,292
Almond milk with omega3-8	27,4456	13,673
Almond milk with omega3-9	26,5148	13,210
Almond milk with omega3-10	27,0378	13,470

Statistical methods used for data analysis were Comparing pairs(fig 2) and Describe-comparative (fig.3), Analyse-it (Microsoft Excel). The results showed a small difference between the two types of almond milk (fig 2). The most values for almond milk with omega 3 phosphorus content were concentrated between 13, 029 to 13, 470 mg/100 g, and between 11, 069 to 11,464 mg/100 g.

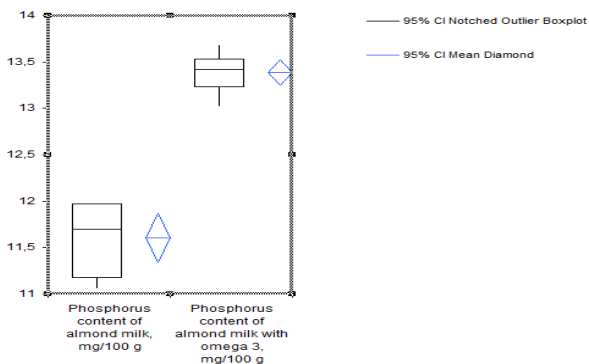


Fig.2. Difference between phosphorus content from almond milk and phosphorus content from almond milk with omega 3

	n	Mean	95% CI	SE	SD
Phosphorus content from almond milk, mg/100 g	10	11,6075	11,3429 to 11,8721	0,11699	0,36995
Phosphorus content from almond milk with omega 3, mg/100 g	10	13,3856	13,2472 to 13,5240	0,06119	0,19349

Fig. 3 Describe –Comparative statistical method for comparing phosphorus content of the two types of almond.

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