

DETERMINATION OF PROTEIN CONTENT IN ALMOND MILK BY COLORIMETRICALLY ASSAY WITH FOLIN CIOCALTEAU REAGENT

Eng.drd. Alina Florentina DAN
Faculty of Agricultural Sciences,
Food Industry and Environment
Protection, "Lucian Blaga"
University of Sibiu



Eng.drd. Anca Maria ȚIFREA
Faculty of Agricultural Sciences,
Food Industry and Environment
Protection, "Lucian Blaga"
University of Sibiu



Ph.D.eng. Ovidiu TITA
Department of Agricultural Sciences
and Food Engineering, Faculty of
Agricultural Sciences, Food Industry
and Environment Protection,
"Lucian Blaga" University of Sibiu



REZUMAT. Scopul studiului a fost determinarea conținutului total de proteine din laptele de migdale prin utilizarea unei metode mai puțin sensibile la medicamente care interferează și sunt mai sensibile la proteine. Două tipuri de probe de lapte de migdale au fost amestecate cu Reactiv 1/Lowry. Amestecul a fost incubat la temperatura camerei timp de 10 minute. După ce reactivul Folin-Ciocalteu a fost adăugat și amestecul a fost incubat timp de alte 30 de minute, conținutul total de proteină a fost analizat colorimetric. Absorbanța a fost măsurată pe un spectrofotometru (Shimadzu) la 750 nm. Curba de etalonare s-a realizat folosind o substanță de referință după cum urmează: 0,025 mg / ml; 0,05 mg / ml; 0,10 mg / ml; 0,15 mg / ml; 0,25 mg / ml. Rezultatele pentru conținutul total de proteine din lapte de migdale s-au situat în jurul valorii de 1,17%.

Cuvinte cheie: lapte de migdale, proteine, Folin-Ciocalteu.

ABSTRACT. The aim of this study was to determinate the total content of protein in almond milk by using a procedure less sensitive to interfering agents and more sensitive to protein. In order to do this, two types of samples of almond milk were mixed with Reagent 1/Lowry reagent. The mixture was incubated at room temperature for 10 minutes. After the Folin-Ciocalteu reagent was added and the mixture was incubated for another 30 minutes, the total content of protein was assayed colorimetrically. The absorbance was measured on a spectrophotometer (Shimadzu Scientific Instruments) at 750 nm. The calibration curve was made using a reference substance as followed: 0,025 mg/ml; 0,05 mg/ml; 0,10 mg/ml; 0,15 mg/ml; 0,25 mg/ml. The results for total content of protein from almond milk were around 1,17 %.

Keywords: almond milk, protein, Folin-Ciocalteu

1. INTRODUCTION

Almond milk is a milky drink made from ground almonds. One of his most important benefits comes from the fact that it does not contain lactose. Some of the people are intolerant to this sugar and if they consume animal milk they can present some of the following symptoms: bloating, diarrhea, discomfort, vomiting. Researchers showed that consuming almond milk instead of animal milk, the behavior of people have visibly improved and those symptoms disappeared(*Iaconoa G, 2008*).

The high content of monounsaturated fatty acids from almond milk has been associated with reducing heart disease and the big amount of vitamin E with antioxidant activity(Jenkins,2002). It is low in

calories and carbohydrates, being advantageous for those trying to lose weight.

It is known that protein content from almond is high. The purpose of this study was to determinate the content of proteins from an almond milk obtained from Spanish almonds milk using a method that doesn't requires long time and, in the same time, being less sensitive to interfering agents.

2. MATERIALS AND METHODS

1. Chemicals and reagents.

The following analytical reagents were obtained from Sigma Co: sodium carbonate, cupric sulfate, sodium potassium tartrate, sodium lauryl sulfate (SLS), sodium hydroxide and Folin Ciocalteu

reagent. Standard materials for protein were obtained also from sigma Co.

The copper reagent was prepared as followed: 20 mg sodium carbonate were dissolved in 260 ml water, 0,4 mg cupric sulfate in 20 ml water and 0,2 mg sodium potassium tartrate in 20 ml water. The three solution were mixed.

For preparing 1 % SLS solution, 1 mg was dissolved in 100 ml water and for preparing 1M solution of NaOH, 4 mg were dissolved in 100 ml water.

Lowry reagent was prepared by mixing sodium lauryl sulfate, 1 % with sodium hydroxide, 1M and cooper reagent in these proportions: 1:1:3(Stoescheck,1990)

The Folin Ciocalteu reagent, 0,2 N was prepared by mixing 10 ml 2N Folin with 90 ml Water and kept in an amber bottle.

2. Colorimetrically assay of total content of protein

10 g almond milk, respectively 10 g of almond milk with olive oil were weight in a 100 ml wolumetric flask and brought to volume with water. Because the concentration could not be estimated , a range of 2-3 dilutions were prepared. The intermediate dilution (3: 25) . was taken in calculations.

Briefly, 5 ml almond milk(indicated dilution), respectively 5 ml of almond milk with olive oil, and 5 ml Lowry reagent were mixed and incubated at room temperature for 10 minutes. Then, 2,5 ml Folin Ciocalteu were added , vortexed and the mixture were incubated for 30 minutes more at room temperature (Lowry,1951).

The absorbance was measured on a spectrophotometer (Shimadzu Scientific Instruments) at 750 nm. For reading absorbance, glass cuvetts were used.The calibration curve was made using a reference substance as followed: 0,025 mg/ml; 0,05 mg/ml; 0,10 mg/ml; 0,15 mg/ml; 0,25 mg/ml.

3. RESULTS AND DISCUSSION

Fourteen samples of two types of almond milk have been investigated: almond milk normally and almond milk with olive oil. The values for reference substance concentration and its absorbance are shown in table 1 and figure nuber 1 shows the calibration curve , where it can be seen that the value of R-squared is 0,9918.

Table 1. Substance concentration and its absorbance

Standard	Concentration of reference substance, [mg/ml]	Absorbance Mean, [nm]	SD	%RSD	Readings
Standard 1	0	0,2851	0,0005	0,16	0,2847
					0,2849
					0,2856

Standard 2	0,025	0,3589	0,0007	0,18	0,3589
					0,3590
					0,3687
Standard 3	0,05	0,4148	0,0005	0,13	0,4153
					0,4142
					0,4148
Standard 4	0,1	0,4949	0,0006	0,11	0,4949
					0,4950
					0,4948
Standard 5	0,15	0,5904	0,0010	0,16	0,5895
					0,5904
					0,5914
Standard 6	0,24	0,7314	0,0008	0,12	0,7315
					0,7316
					0,7312

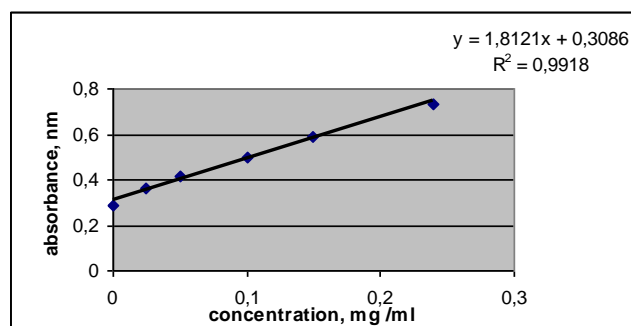


Fig.1 Calibration curve and standard equation for reference substance

Table.2. Almond milk samples and their total content of protein

Sample number	Total content of protein determinated from almond milk,%	Total content of protein determinated from almond milk with olive oil,%
Sample 1	1,183	1,134
Sample 2	1,181	1,124
Sample 3	1,182	1,137
Sample 4	1,177	1,130
Sample 5	1,179	1,124
Sample 6	1,181	1,132
Sample 7	1,178	1,133
Sample 8	1,182	1,126
Sample 9	1,178	1,137
Sample 10	1,180	1,119
Sample 11	1,181	1,141
Sample 12	1,182	1,117
Sample 13	1,182	1,135
Sample 14	1,181	1,124

Table 2 shows the values determined with calorimetrically assay with Folin Ciocalteu reagent for every sample. The values are expressed as percentage.

One of the statistical method used for data analysis was Summary (Microsoft Excel-Analyze-it, figure 2 and figure 3). This analysis shows the number of observations analysed and summary statistics.

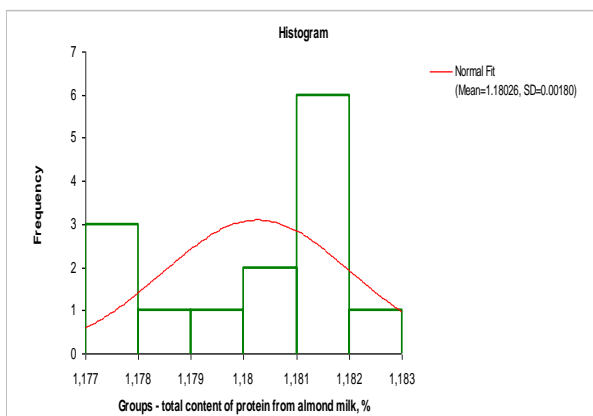


Fig.2. Histogram for total content of protein from almond milk, %

As it can be seen from figure number 2, the mean value for total content of protein from almond milk were 1,18026%. The most values were between 1,181 and 1,182 %.

Regarding total content of protein from almond milk with olive oil, figure number 3 shows that the most values were between 1,13 and 1,135 %. The mean value for protein content in this case was 1,12948 %.

The results showed a insignificant difference between the two type of almond milk. It can be seen that the total content of protein from almond milk with olive oil was smaller than the value of total content of protein from normaly almond milk. This can be due to interfering proteins with almond milk fats during the procedure.

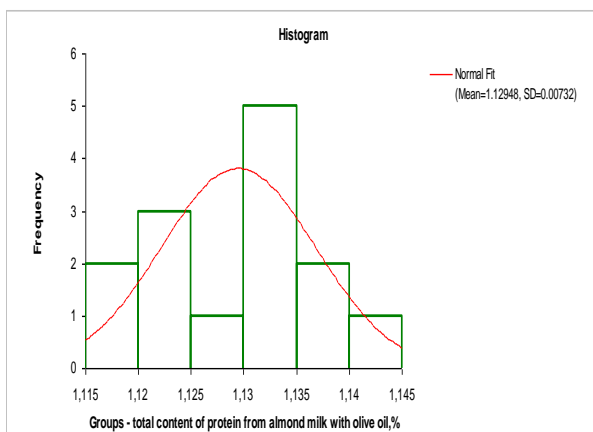


Fig.3. Histogram for total content of protein from almond milk with olive oil, %

In order to compare the values of the two groups t-test(Median test) was used as a statistical method (Microsoft Excel-Analyze-it, Table 3). The test showed that the mean difference is 0,05079, insignificant for this types of almond milk.

Determination of total content of protein from almond milk by colorimetrically assay with Folin Ciocalteau reagent proved to be a quick method.

Table 3. T-test applied to compare the values of the two groups.

n	28			
Groups	n	Mean	SE	SD
Total content of protein from almond milk, %	14	1,18026	0,000481	0,00180
Total content of protein from almond milk with olive oil, %	14	1,12948	0,001956	0,00732
Mean difference	0,05079			
95% CI	0,04665 to 0,05493			
SE	0,002014			
t statistic	25,22			
DF	26,0			
2-tailed p	<0,0001			

Recording absorbance for this method needed only be done within 10 minutes of each for this procedure. This procedure demonstrated to be less sensitive to interfering agents and more sensitive to protein.

4. ACKNOWLEDGMENT

This study was made possible through the project "Romanian Research Integration in European Research Context, POSDRU/88/1.5/S/60370", financed from European Social Fund by Operational Sectoral Programme for Human Resources Development 2007-2013.

5. REFERENCES

- [1] Iacono, G., Lospallutia, M.L., Licastro, G., Scalicia, C., A new formula based on almond milk next term for management of cow previous term milk next term intolerance and food allergies, Gastroenterologia Pediatrica, Palermo, 2008
- [2] Jenkins, D.J., Kendall, C.W., Marchie, A., Parker, T.L., Connelly, P.W., Qian, W., Haight, J.S., Faulkner, D., Vidgen, E., Lapsley, K.G., Spiller, G.A., Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial., Circulation, Journal of the American Heart Association, ;106(11):1327-32, 2002
- [3] Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. J. Biol. Chem. 193:265, 1951
- [4] Stoescheck, C.M. Quantitation of Protein. Methods in Enzymology 182: 50-69, 1990