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INFLUENCE OF PARTICLE SIZE AND SAMPLE-EXTRACTION MIXTURE RATIO ON EXTRACTION OF LUTEIN FROM LAYING HENS FEED MIXTURE

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INTRODUCTION

The role of lutein in human and animal health has already been investigated. [1,2] Due to its importance, it is of great interest to determine its content in the food we consume or is consumed by animals whose products we use in our diet. In this study, the influence of particle size and dry matter (sample):extraction mixture ratio on determination of lutein content in laying hens feed was investigated. For sample preparation modified standard method was used. [3] All extractions were performed on the same samples and assisted by direct ultrasonic extraction. The lutein content of the samples was determined by means of HPLC with a UV-VIS detector at 450 nm.

EXPERIMENTAL

Samples
All used samples was in form available on the market or grinded on a household grinder, sieved and stored in a cooler at 4 °C.

Reagents
Methanol, acetone, 2,6-Di-tert-butyl-4-ethylphenol (BHT), potassium hydroxide, hexane, ethyl acetate, tetrahydrofuran. Reagents used in this investigation was HPLC grade.

Apparatus
Extractions were assisted by ultrasonic homogenization system (Sonoplus 3100, Bandelin, Berlin, Germany) with probe VS 70 T. For determination of lutein content in samples, a high-performance liquid chromatograph (Shimadzu) was used along with a Shim-pack GIST C-18 column (150 mm x 4.6 mm, 5 µm), Shimadzu, centrifuge (Rotina 380 R, Hettich) and rotavapour.

Sample preparation method
In amber cuvetts 1 g of hens feed mixture were weighed, and extraction mixture which consists of a mixture of methanol and acetone and 0.2% solution of BHT in methanol was added. Extraction was assisted by ultrasonic homogenization system during 3 minutes with pulse interval 3 s, time between pulses was 2 s and amplitude 70%. If extraction was performed with saponification, in extraction mixture was added 40% methanolic solution KOH (Figure 1). After extraction, samples were left in the dark for 10 minutes and centrifugated for 10 minutes 6000 rpm. 1 mL of supernatant was filtrated with syringe filter 0.45 µm and evaporated under vacuum. By adding 1 mL of hexane:ethyl acetate mixture samples were ready for HPLC analysis. Mobile phase used for HPLC analysis consisted of methanol:THF=9:1.

RESULTS AND DISCUSSION

Influence of particle size and saponification

Table 1. Comparison of lutein content obtained in ungrinded and grinded samples

sample	mg/L (ppm)	mg/kg feed mixture	average	SD	RSD (%)	confidence(±)
1	0.14905	2.981	**2.987**	0.0722	2.417	0.071
2	0.17664	3.533				
3	0.14991	2.998				
4	0.15360	3.072				
5	0.14481	2.896				
1 grinded hens feed mixture	0.21834	4.367	**4.546**	0.324	7.123	0.284
2 grinded hens feed mixture	0.23383	4.677				
3 grinded hens feed mixture	0.22742	4.548				
4 grinded hens feed mixture	0.21344	4.269				
5 grinded hens feed mixture	0.24997	4.999				

** the result marked in red is not included in the statistics*

Although the RSD value in grinded samples was significantly higher than in ungrinded samples, determined lutein content is 52% higher in grinded samples (*Table 1*). Therefore all following analysis were performed in grinded samples.

Influence of saponification

Comparing the results obtained with grinded samples by the same method but with saponification, 3% more lutein was determined with saponification and the RSD value was significantly reduced (from 1.490% to 0.420%).

Influence of sample-extraction mixture ratio

Table 2. Comparison of various sample:extraction mixture ratio

extraction mixture	sample - extraction mixture ratio	mg/L (ppm)	mg/kg feed mixture	RSD (%)
methanol: acetone = 1:1	1:5	0.63030	3.152	4.513
methanol: acetone = 1:1	1:10	0.39565	3.956	2.651
methanol: acetone = 1:1	**1:20**	**0.25741**	**5.148**	**1.498**
methanol: acetone = 1:1	1:40	0.05039	2.015	5.285

The ratios of sample:extraction mixture: 1:5, 1:10, 1:20 and 1:40 were investigated, and the obtained results (3.152; 3.956; 5.148 and 2.015 mg / kg of sample) indicate that the best ratio is 1:20. Along with the highest determined lutein content, this ratio also showed the lowest RSD value (Table 2).

Figure 1. Apparatus for direct ultrasonic extraction (Sonoplus 3100, Bandelin, Berlin, Germany)

CONCLUSION

In this research, a modified standard method was used. The best results (highest lutein content determined and lowest RSD value) were obtained using grinded and sieved samples with saponification, sample:extraction mixture ratio of 1:20 and direct ultrasonic extraction. Although the obtained results are satisfactory, the modified method requires additional tests in order to obtain even better results.

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