TURBIDIMETRIC DETERMINATION OF RAW FAT IN CROP SEEDS

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ABSTRACT - *A variant of a turbidimetric method of raw fat determination from seed, which proved to be simple, fast, and accurate, was described in this paper. The method was based on the extraction of lipids from 20 mg samples into acetone followed by their treatment with 1.5% solution of sulfosalicylic acid and spectral measurement at 440 nm against a blank of the reagents. Suitable volumes of acetone extracts should be taken for oily seed samples such as flax, sunflower, or soybean. A standard curve was made with raw fat extracted from the species being analysed. For analysing a large number of samples, the standardization of the turbidimetric method with a few representative Soxhlet values was recommended. Possible interferences, as well as the real results obtained within large scale analyses ,are also shown.*

Key words: raw fat, turbidimetric assay, plant seeds

INTRODUCTION

Lipids are major building blocks of cell membrane, as well as a food quality parameter (Mou et al., 2000). In our previous works (Drochioiu, 1999, 2005), we have demonstrated the feasibility of raw fat determination in seeds with a turbidimetric method, based on acetone extraction and lipid reaction with trichloroacetic acid or with sulfosalicylic acid.

Acetone has already been shown as a potential solvent for Soxhlet extraction. However, in spite of its toxicity and inflammability, hexane is the most usually used for oil extraction from oilseeds. Unfortunately, conventional Soxhlet-type extraction uses flammable solvents and is time-consuming. Furthermore, the oil content is determined gravimetrically after solvent

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evaporation. The classical procedures based on solvent extraction require several grams of dried sample and, consequently, determination is a slow process. In addition, they are less suitable for routine analysis when series of small amount samples have to be analysed.

Hence, refractometry and gas chromatography, after oil extraction in organic solvents, are also used (Levy, 1994). A quicker procedure, based on supercritical fluid extraction, was developed for determining oil, fat and lanolin present on raw wool fibres. This method also requires several grams of sample. The lipid concentration measurement based on aggregating property of a lipid in a supporting fluid by absorption of a labelled lipid was also proposed (Ilekis, 1983). Some procedures for the determination of lipids are based on dispersion of liquid sample by treating a sample with a surfactant in an aqueous medium and measuring light absorbance in an ultraviolet region (Kawaguchi et al., 1996). However, these procedures are not suitable for small amounts of dried seed flour.

Therefore, a micro-assay is proposed on raw fat from the crop seed samples, based on acetone extraction under sonication in Eppendorf vials, followed by the treatment with a sulfosalicylic acid solution.

MATERIALS AND METHODS

Apparatus. A UVIKON 933-KONTRON double beam UV/VIS spectrophotometer equipped with glass cells was used for the spectral measurements. All chemicals were reagent grade and used as supply. Sulfosalicylic acid was purchased from Sigma (Deisenhofen, Germany) and acetone, from Chimopar (Bucharest).

Biological Materials. Seeds of maize (*Zea mays*), wheat (*Triticum vulgaris*), rye (*Secale cereale*), and flax (*Linum usitatissimum*) belong to the collection of Gene Bank of Suceava, Romania.

Procedure. Seed samples with a residual humidity of 12–14% were milled into a fine powder. Then, duplicates of 20 mg flour of analysed samples were introduced in Eppendorf vials and extracted with 1.0 mL of acetone. Thus, the vials were capped, sonicated for 5 min, and centrifuged. Volumes of 200 μL of maize supernatant were pipetted into the colorimeter tubes and 1.8 mL of 1.5% sulfosalicylic solution was added. If the samples are rich in oil, the supernatant volume is accordingly diminished (20 μL was treated with 2 mL sulfosalicylic solution). The supernatant volume may increase up to 200 μL in the case of wheat, barley, and rye. The mixture was shaken vigorously and after standing for minimum of 30 min, the absorbance of each sample was read at 440 nm, in 1-cm path glass cells against a blank (a mixture made of 20-200 μL acetone and 1.8 – 2.0 mL 1.5% sulfosalicylic acid). The raw fat concentration was calculated from the standard curve made with the representative values for each lipid. Calibration curves were made with lipids extracted with petroleum ether in a Soxhlet

apparatus, and gravimetrically determined. The stock solutions were prepared in acetone. They were kept in cool and dark places for at least two weeks.

Statistics. Results obtained were processed statistically by calculating deviation (d), root-mean-square error (s), standard deviation of the mean (s_d), correlation coefficient (r), coefficient of variation, confidence level (α), as well as t parameter.

RESULTS AND DISCUSSION

The absorbance of the resulting turbidity was measured at 440 nm, being proportional to the concentration of raw fat in the sample. Acetone has extracted thoroughly and quickly the lipids from seed and, therefore, the extraction could not be a limitation for the accuracy of determination. In addition, lipid solutions resulted in a maximum turbidity when treated with a 1.5% sulfosalicylic acid solution. As for flax, the turbidity at 440 nm of the mixture of lipid-sulfosalicylic acid solution increased quickly at the beginning of the reaction presumably due to the inclusion of air bubbles into the mixture. Then, it decreased slowly in the first 30 min and remained almost constant for about 5 hours.

Under the experimental conditions, the free fatty acids in the samples showed no remarkable turbidity. For example, old samples of maize flour, especially those with higher moisture, provided up to one-third of the absorbance of fresh samples. The observation was explained by the hydrolysis of lipids in seeds and flours, which afforded glycerol and fatty acids.

20 mg of standard sample of maize flour with 4.0% raw fat (as determined by the Soxhlet method) were treated with 1 mL acetone, and then 200 μL of extract were mixed with 1.8 mL reagent solution. The 440 nm absorbance of the suspension was measured to be 0.605. In all cases, acetone extract should have no more than 500 μg/mL raw fat, and consequently, the final lipid concentration of the suspension had to be less than 100 μg/mL.

In the case of 5 flax samples, a highly significant correlation was found between the raw fat content, determined by Soxhlet method, and the values obtained by the turbidimetric assay (r = 0.974). The differences between the values were found to be not significant at s_d = 2.43 and t = 0.295. Consequently, a regression equation (y = 0.892x + 4.3333), was calculated, where y = raw fat by Soxhlet procedure, and x = the value for oil in flax by the turbidimetric one (*Table 1*).

High correlation coefficients were obtained for all types of seeds, showing a good relationship between raw fat concentration and lipid turbidity in the presence of sulfosalicylic acid. *Figure 1* shows that the oil from samples could be determined accurately by using a standard curve derived from the known samples. In this case, the turbidity of some samples was read, and then oil content of 4–8 samples was determined by Soxhlet procedure. Consequently, a regression

equation, y = 31.509x - 23.24, was calculated, where y = raw fat concentration (Soxhlet) in flax flour, expressed as percentage of dry matter, and x = the absorbance at 440 nm in 1-cm glass cells (*Figure 1*). A highly significant correlation between the raw fat content and the turbidity of the samples was observed (r = 0.989). For this absorbance interval, one can calculate the following regression equation: y = 0.022x – 0.685, where y = absorbance at 440 nm, and x = lipid concentration in opalescent solution (expressed as μg/mL). However, the calibration curve was not linear and possibly better results could be obtained using another absorbance range.

Table 1

Oil content of some flax samples as determined by the proposed turbidimetric procedure

Flax Variety	Oil (% in DM) Proposed method	s^2	s_x	Oil (% in DM) Soxhlet method
Mathis 214	32.30 ± 1.41	0.32	0.33	33.80
Blue D	33.80 ± 1.61	0.42	0.37	33.49
Pinioda	33.70 ± 1.66	0.45	0.38	34.30
Estanzuela	40.20 ± 1.33	0.29	0.31	40.72
Culbert	44.10 ± 1.53	0.38	0.35	44.70
Muscel	45.20 ± 1.29	0.27	0.30	44.65
Redwood	45.60 ± 1.19	0.23	0.27	45.80
Dana	46.70 ± 1.46	0.35	0.34	45.81

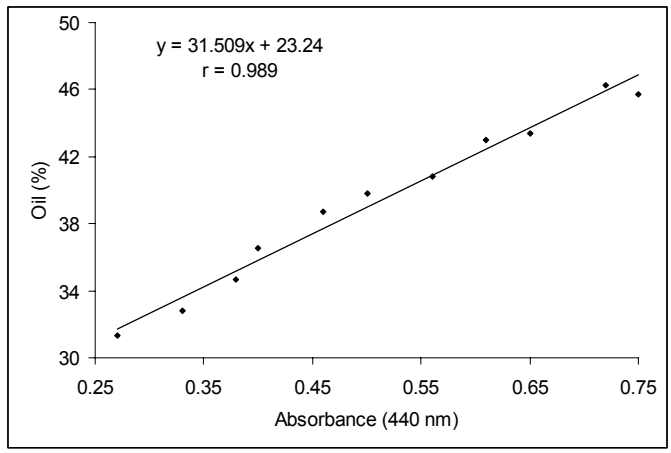

Figure 1- Correlation between the oil content of flax samples and the absorbance of the turbidity of lipid-sulfosalicylic acid system

Nevertheless, a calibration curve with pure flax oil was also plotted (*Figure 2*). Unfortunately, an almost linear shape was found in the range 10–40 μg/mL oil.

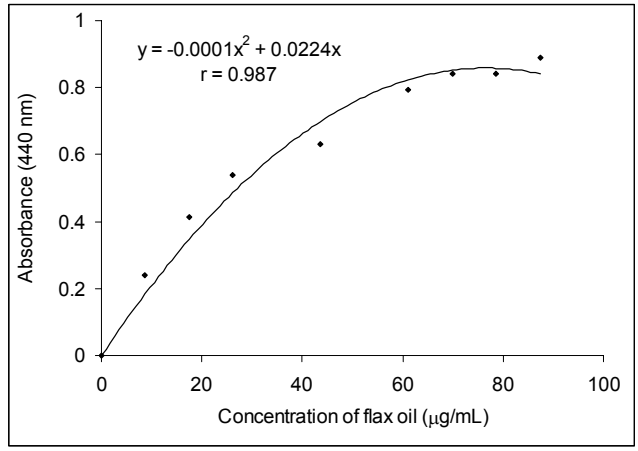

Figure 2- Calibration curve for oil determination in flax

Effect of extraction time on the turbidity was investigated by using a sample of maize flour with 5.6% raw fat. Turbidity proved to be nearly constant for an extraction period of 1–60 min. In addition, the coarseness of the flour seemed not to interfere with the extraction. Oil-rich samples showed the same ease in releasing the lipids in the presence of acetone. Even 1 min extraction time proved to be sufficient for lipid determination in flax. Reproducibility of the method was as good as that of the conventional Soxhlet method only for high sample size, due to heterogeneity of the flours. The turbidimetric method also proved to be effective in quantifying the fat raw content of small samples of seeds, similar to the Soxhlet extraction, without using large amounts of solvent and requiring small amounts of sample. This highly sensitive method could be used for lipid determination in samples of less than 5 mg of seed flour and could be analysed with high accuracy by the turbidimetric method. The proposed procedure could be used to determine the lipids in very small samples and to assess their variability, with respect to their lipid content. The specificity of the acetone method seemed to be dependent on the type of oil in the analysed sample. The reproducibility of this method was dependent upon particle size of the sample. In order to perform a reproducible determination, we recommended that the seeds be milled to a fine powder.

The lipid content of 50 maize samples analysed by the turbidimetric procedure ranged between 3.40 and 6.25% as dry matter. A high correlation coefficient between representative absorbance values and the corresponding 12 raw fat content values was calculated (r = 0.973). Other statistical parameters such as s^2 = 2.75, s_d = 0.74, t = 0.262 and d = 0.24 demonstrated that differences between the two series of values were not significant. The oil content of 46 flax samples was 32.40–46.50%. For eight representative samples the following statistical parameters were calculated: d = 0.59, s^2 = 29.06, s_d = 2.41 and t =

0.245. Therefore, the two series of values did not differ significantly for $\alpha = 0.95$, for which a correlation coefficient, $r = 0.989$, was calculated. The lipid content of wheat and rye samples ranged from 1.65 to 2.25% on a dry matter basis.

The efficiency of the turbidimetric method was higher when compared to the Soxhlet method, especially when a large number of small samples needed to be analysed. The proposed assay could be applied to screen oil-rich varieties in the breeding work or to follow the fat accumulation within the maturing seeds.

CONCLUSIONS

The feasibility of a micro-method for fast determination of raw fat in seeds has been demonstrated. The proposed method is simple, accurate, economic, selective and sensitive, fast, specific, reliable, and of high productivity. It uses small amounts of solvent and sample (20 mg), is not affected by the water content in the sample. Results obtained with this simple and rapid turbidimetric method using acetone and a sulfosalicylic acid solution and the Soxhlet method are in good agreement. The proposed procedure can be used in screening works and to follow lipid accumulation in maturing plants.

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