The Effects of Seed Pretreatments and Extraction Conditions on the Amount of Minor Components in Seed Oils. I. Lipid Peroxides

L.-Å. APPELQVIST, Chemical Department, Swedish Seed Association, Svalöf, Sweden

Abstract

Procedures are described for careful extraction of rape and mustard seeds resulting in oils with no detectable peroxides (<0.02 meq/kg) if high quality viable seeds are used. Oils from heat-damaged seeds contain peroxides even after very careful extraction of the seeds. The effects of separate precautions such as prewashing of equipment and the exclusion of light and heat on the peroxide value of the oil extracted are shown to differ, depending on the kind and quality of seed. Oils from white mustard seed obtain lower peroxide values than oils from rapeseed when subjected to pro-oxidative conditions during extraction.

Introduction

As part of an extensive program aimed at raising the quality of the rape, turnip rape and white mustard seed harvested in Sweden (2), studies were initiated on the effect of seed quality on the peroxide content of the extracted oil. Because the peroxide value of crude rapeseed oil is of primary importance for the flavor stability of the refined and deodorized product, the variation with species as well as different agricultural practices and storage conditions were included in the program. At an early phase of the work it was found, however, that the seed pretreatment and extraction conditions exert a great influence on the peroxide value obtained. No official methods seem to exist, however, for such an analysis (1,4,6,7); e.g., in the AOCS official and tentative methods, the determination of all components in the oil except free fatty acids start with the oil and not with the seed. The present paper will describe some factors affecting the peroxide value of the oil extracted from viable, high-quality as well as heat-damaged seed of rape and white mustard. Following papers will deal with the content of chlorophyll and phosphatides of a seed oil as affected by extraction conditions.

Materials and Methods

Seeds

Winter rapeseed *(Brassica napus)*, variety Svalöfs Matador, was harvested when fully mature. One portion was carefully dried to obtain a sample with a high germination capacity (94%), and another portion was dried with hot air to get a sample with low viability (1%). The samples were stored at 6–7% in closed containers at 25C for 7–8 months before being used.

White mustard seed *(Sinapis alba)*, variety Svalöfs Seco, was treated in a similar manner and the two samples showed 82% and 0% germination capacity, respectively. All samples had a low content of free fatty acids when put in storage (0.2–0.3%) and also after 7–8 months (0.3–0.6%). The seeds were free of dockage and were carefully mixed before sampling for each analysis.

Chemicals, etc.

Phillips 66 Hexane, redistilled before use (bp 68–70C); chloroform (Fischer) analytical reagent grade; glacial acetic acid (Mercks) analytical reagent grade; potassium iodide (May and Baker) analytical reagent grade; starch solution, 1% of Mercks analytical reagent grade soluble starch in water.

Cotton wool and filter papers were defatted by Soxhlet extraction with reagent grade chloroform-methanol (3:1) for 6–8 hr.

Seed Pretreatments and Extracting Conditions

Methods A–F. Extraction in stainless steel tubes as described by Troeng (12) and Appelqvist (3).

A. Ordinary cleaned steel tubes and steel balls were repeatedly washed with hexane. The stoppers were placed in hexane solution and air-dried. Nitrogen was flushed through the tubes, when kept in a vertical position, for 10 min. The seed samples, about 15 g, with natural moisture contents (6.4% and 5.2% for viable and dead rape and 7.3% and 5.8% for white mustard, respectively), were added and the nitrogen flush continued for another 10 min. Thirty-five milliliters of hexane, which had been set under vacuum at room temperature in order to free it from dissolved air, was added and the hexane-washed petrol-resistant rubber stoppers were fastened in the tubes, which were placed in special boxes (9) and shaken in a horizontal position for 1 hr. The contents were filtered through defatted filter paper and hexane-washed glass equipment in a dark room. The tubes were washed with 2–3 portions of 5 ml hexane each and these washings filtered into the same Erlenmeyer flask as the main solution. The clear solutions were evaporated at maximum of 30C in a vacuum oven overnight. Nitrogen was allowed to enter the vacuum oven upon release of vacuum. The flasks containing the oils were placed in light-protected desiccators and weighed in a dark room, in order to obtain the oil content. The solvent mixture for the peroxide determination was added and the peroxide content determined.

B. Identical to A except that deaeration of the hexane and nitrogen flushing of the tubes was omitted.

C. As A with the following exceptions: Extra washing of all glassware and tubes was omitted. Ordinary filter paper was used instead of defatted.

D. As B except for the exclusion of daylight.

E. As D but with solvent evaporation on a hot plate and in a hot ventilated cabinet for 2.5 hr as in the ordinary multi-sequential method for oil determination (9).

F. As A but the seeds were dried at 100C overnight before being extracted.

Methods G and H. Extractions in Butt-type apparatus.

G. The extraction was performed as described for soybeans in AOCS method Ac. 3-44 (1) with the following exceptions. The whole seeds were not ground in a mill but in a mortar. The specifications for

petrol ether (AOCS H. 2-41) were not checked. The petrol ether solution of the lipids after the second extraction was stored in the refrigerator overnight before evaporation. When nearly solvent-free, the lipid solutions were transferred to Erlenmeyer flasks, in which the evaporation was completed. The peroxide value was determined immediately after a constant weight of the flask was obtained.

H. Extraction as in G with the following precautions added: No drying of seed before grinding, washing all glass equipment with hexane immediately before use, using defatted filter paper, excluding daylight from the extraction room, evaporating the oil solution in vacuum at 30C, and finally keeping the oil received in the flasks in desiccators protected from daylight until weighed and dissolved in chloroform-acetic acid.

Peroxide value was determined according to AOCS method Cd-8-53 (1) except that the thiosulfate was 0.002 N. The result was expressed as meq peroxide/kg oil.

Results and Discussion

Three single determinations were made on each sample except one. The results are shown in Table I.

From this it is obvious that it is possible to obtain an oil with a peroxide value which is very low, or completely zero, if high-grade seed is used and a series of precautions are taken during the extraction process (conditions A and B). Upon addition of starch solution to the sample no blue color could be observed with such samples where a peroxide value of 0.00 is shown in Table I. As a peroxide value of 0.05 was easily seen we consider the peroxide value zero to be less than 0.02 in our experiments. On the other hand, even then heat-damaged, nonviable seeds will give measurable peroxide values. This could be due to in situ oxidation or a very high susceptibility to oxygen during the extraction procedure. As flushing of the extraction tubes with nitrogen prior to disintegration had no effect (A compared to B) it seems probably that an in situ oxidation had occurred.

A comparison between B and H also gives support to the theory of performed peroxides in the seeds, as extractions, although filled with precautions, should be different enough to raise the peroxide content of the oils from damaged seeds to different levels in the two procedures if an oxidation really occurred during the extraction.

The nearly identical values obtained with and without nitrogen flushing of the tubes makes this precaution unnecessary. Nitrogen flushing was tried, however, in an effort to obtain the lowest possible peroxide values from damaged seeds.

Method C clearly shows the benefit of hexane washing of equipment before use. Numerous earlier results in our laboratory have shown that unwashed equipment can give as low peroxide values in one determination as is obtained with prewashed equipment, while the duplicate will be fairly high.

Method D compared to A shows the necessity of excluding daylight from the extracted oil before peroxide determination.

The figures obtained with method E clearly shows the importance of evaporation of solvent in vacuum at low temperature.

Method F compared to A shows the destruction of peroxides when the seeds are heated prior to extraction, a procedure frequently used in determinations of oil content of oil seeds.

Considering now the two versions of conventional extraction (Butt-type apparatus) it is obvious that method H, where a number of precautions were taken, will give peroxide values as low as methods A and B. Also, the standard errors for A, B and H are similar.

Method G, which is the official AOCS method for determination of oil content in soybeans (with the few modifications stated above), gives fairly high peroxide values. However, it should be observed that in this method the seeds were heated to 130C for 2 hr before grinding so the peroxides determined must have developed during the grinding, extraction and evaporation processes.

Since extraction in the steel tubes is more convenient than in Butt-type extractors, they were used to study the separate changes in procedure, viz., methods B, C, D and E.

As a result beyond the scope of the investigation, the differences in peroxide values between rape and white mustard oil when subjected to the pro-oxidative conditions in methods E and G, is of great interest. Thus the undamaged as well as the heat-damaged white mustard seed, will develop lower peroxide values than the comparative rape samples although they have similar fatty acid patterns. This observation is supported by findings in commercial crude oils from rape and white mustard, where the latter shows a higher oxidative stability (13).

Methods E and G yield much higher peroxide values for the heat-damaged seeds than for the undamaged ones and in this sense provides a measure of oxidative stability. However, a more valuable approach from the point of view of edible oil technology is to make two separate determinations: 1) The peroxide value of the in situ seed oil in order to establish the existing status of the oil and 2) the oxidative stability of the oil obtained by careful extraction. Such a stability measurement should be made by standard methods designed for this purpose and not by exposing the sample to variable pro-oxidative conditions as is the case with methods E and G. The two sets of data so obtained would be of value in controlling and improving oil seed quality.

In studies on oil seed quality, the peroxide value

TABLE I

Peroxide Values Expressed as meq/kg in Oils from Rape and White Mustard Seeds Pretreated and Extracted in Various Ways

Sample	Method of analysis[a]							
	Steel extraction tubes						Butt-type extractors	
	A	B	C	D	E	F	G	H
Rape, undamaged	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.17	0.07 ± 0.05	5.85 ± 0.48	0.00	4.54 ± 0.21	0.00 ± 0.00[b]
Rape, heat damaged	0.52 ± 0.05	0.54 ± 0.02	0.53 ± 0.12	1.58 ± 0.08	8.48 ± 0.38	0.00	6.95 ± 0.25	0.58 ± 0.06
White mustard, undamaged	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.10	0.00 ± 0.00	1.91 ± 0.80	0.00	1.26 ± 0.06	0.00 ± 0.00
White mustard, heat-damaged	0.59 ± 0.06	0.63 ± 0.07	0.57 ± 0.05	1.47 ± 0.08	4.68 ± 0.78	0.00	4.56 ± 0.12	0.77 ± 0.08

[a] All values reported represent the mean of three single determinations ± standard error except for the sample marked [b] in which case only two samples were analyzed.

of the seed oil is seldom stated. Davis (5) however reported on the peroxide value of the oil extracted from three varieties of peanuts stored at different moisture contents. As the beans were heated to 130C for 1 hr before grinding and as no precautions were described, the figures obtained can hardly reflect the oxidation status of the oil in the stored beans.

Rutkowski and Makus (10) studied the changes in peroxide value when rapeseeds of different degrees of maturity were stored for up to 5 months at 18–20C at a relative moisture content of 60%. Their figures, viz. 1.2–3.2 meq O_2/kg in the fully ripe seeds are considerably higher than ours obtained by methods A, B and H. Nothing is stated, however, about the methods used either in that paper or in another by the same authors (11) where they report on the peroxide values of stored rapeseed, which was either undamaged, mechanically damaged or mould infested.

Janíček and Pokorny (8), in their study on the effect of the conditions of storage of rapeseed on the quality of the oil extracted, likewise state no precautions taken during the extractions except for vacuum evaporation of the lipid extract. Although they state that the rapeseed used was "of first quality" no figures for germination percentage were reported. The peroxide values they obtained from storage conditions comparable to ours (their No 5) are fairly high, viz. 4.7–7.6. They conclude that the peroxide values change irregularly during the storage of the seeds.

As the many precautions in our methods B and H result in a low analysis capacity it is of practical interest to include as few precautions as possible in the extraction procedures with either the steel tubes or the Butt-type apparatus. A careful washing of the equipment with hexane as well as vacuum evaporation of the solvent from the lipid extract, and protection of the oil from daylight are precautions shown to be necessary by the present investigation. Further studies are needed to determine if minor simplifications can be made. As petroleum fractions are the usual lipid solvents used for extracting oil seeds, hexane was used in these investigations. Preliminary studies with chloroform as the extraction solvent yielded higher peroxide values than obtained with hexane. Therefore also the effect of different solvents on the peroxide value of the lipids extracted will be further studied.

ACKNOWLEDGMENTS

The financial support from Svenska Extraktionsföreningen, Karlshamn, Sweden, and Malmfonden, Swedish Council for Scientific Research and Industrial Development. Technical assistance by Mr. S.-Å Johansson and Miss E. Jönsson.

Communication from the Swedish Seed Association, No. 265.

REFERENCES

1. American Oil Chemists Society, Official and Tentative Methods (1945–).
2. Appelqvist, L.-Å., "Quality Problems in Cruciferous Oil Crops," In Åkerberg and Hagberg. Ed, Recent Plant Breeding Research, Svalöf, 1946–1961; published, Almqvist & Wicksell, Stockholm, 1963, p. 301–324.
3. Appelqvist, L.-Å., JAOCS *44*, 209–214 (1967).
4. British Standards Institution, British Standard Specifications, Vegetable Oils, 1961, London.
5. Davis, N. D., JAOCS *38*, 516–517 (1961).
6. Deutsche Geselischaft für Fettwissenschaft, DGF-Einheitsmetoden (1950–), Wissenschaftliche Verlagsgesellschaft, Stuttgart.
7. International Union of Pure and Applied Chemistry, Standard Methods of the Oils and Fats Division of the I.U.P.A.C., 1964, Butterworths, London.
8. Janícêk, G., and J. Pokorny, Scientific Papers from Institute of Chemical Technology, Prague, Food Technol. 7(2), 173–179 (1963).
9. Lindberg, J. E., S. Troeng and G. Lyttkens, Sveriges Utsädesfören. Tidskr. *75*, 281–301 (1956).
10. Rutkowski, A., and Z. Makus, Oleagineux *13*, 203–205 (1958).
11. Rutkowski, A., and Z. Makus, Fette Seifen Anstrichmittel *61*, 532–535 (1959).
12. Troeng, S., JAOCS *32*, 124–126 (1955).
13. Holm, U., personal communication to the author.

[Received May 2, 1966]