Comparison of Methods for the Analysis of Phosphorus in Canola Oils[1]

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ABSTRACT

Three chemical and two instrumental methods for determining phosphorus in crude and refined canola oils were compared with the official AOCS method. Of the three different digestion-spectrophotometric procedures examined, the oxygen bomb combustion method appeared to offer the best combination of speed, accuracy and precision. In contrast, the perchloric acid procedure was faulted for poor agreement with the AOCS procedure and poor reproducibility between batches, whereas the saponification procedure was very labor intensive and exhibited poor precision. Of the two instrumental methods examined, atomic absorption spectrophotometry was found to be more sensitive than molecular emission cavity analysis although both procedures were reported to be unsatisfactory in detecting inorganic phosphorus.

INTRODUCTION

The AOCS method for phosphate determination is not widely used because it is relatively cumbersome and lengthy. Alternative procedures (1-4) are often chosen because of greater speed or smaller sample size than the official method. In this study, we have examined several methods which are being used in Canada. The procedures were compared with the official AOCS method in order to determine their accuracies relative to the AOCS method as well as their advantages and disadvantages.

MATERIALS AND METHODS

Twelve samples of canola oil were analyzed in this study including 3 samples each of crude, degummed, caustic refined, and refined and bleached oils which had been collected from Western Canadian Canola Crushers and from Canada Packers Oil Refinery (Toronto). Canola is a trademark of the Canola Council of Canada and refers to seed, oil, and meal from varieties of *Brassica campestris* and *Brassica napus* which have low levels of erucic acid and glucosinolates. The samples were analyzed by the AOCS procedure at all 3 laboratories in the study, but due to lack of equipment and expertise, the other procedures were carried out at one laboratory only.

AOCS Procedure

The procedure followed was basically as described in the AOCS Official Method Ca 12-55 (5). Three grams of sample and 0.5 g of zinc oxide were measured into a crucible and heated on a hot plate to char the oil. The crucible was then placed in a muffle furnace and heated from 450 to 580 C until completely ashed. The ash was dissolved in water and HCl, was neutralized with KOH and then re-acidified and made up to 200 mL. An aliquot was treated with molybdate and reducing agent to form molybdenum blue. The color was read at 650 nm using a spectrophotometer, and the phosphorus content was determined by comparison with a standard curve prepared from solutions of NaH_2PO_4.

Laboratory B used the colorimetric procedure described by the AOAC (6) in which the phosphorus is converted to molybdovanadophosphate.

Perchloric Acid Digestion

The procedure followed was based on the procedure described by Rouser et al. (7) for phosphorus in thin layer chromatographic (TLC) scrapings. A 10% solution of the oil in hexane was accurately prepared. This allowed accurate sampling of 20 mg using a 0.2-mL pipette. The sample (0.2-mL) in a 16 mm × 100 mm screw-capped tube was placed in a heated block (100 C) for 15 min to remove the hexane. After cooling, 0.4 mL of a mixture of H_2SO_4 and $HClO_4$ (4:1) was added to each tube and the tube was placed in a block heater at 180-200 C. The fumes from the tubes were vented to a water aspirator. After 30 min digestion, the sample was cooled and 5 mL H_2O, 1 mL of 2.5% ammonium molybdate and 1 mL of 10% ascorbic acid was added. The tube was then heated at 100 C for 20 min and the blue color was measured at 650 or 750 nm, depending on the phosphorus content. The phosphorus content was estimated from calibration curves prepared from NaH_2PO_4.

Oxygen Bomb Ashing

The procedure followed was as described by Yeun and Kelly (1). A sample of oil (0.4-0.8 g) was added to the oxygen bomb sample cup along with zinc oxide (0.03-0.04 g). The capsule was heated briefly on a hot plate to disperse the zinc oxide. Water (1.2 mL) was placed in the oxygen bomb along with the sample cup and the sample was burned. The residue was dissolved and removed from the bomb with the aid of dilute HNO_3 and was filtered into a 25-mL flask. Phosphate was determined by the molybdovanadophosphate procedure (6).

Saponification Procedure

The procedure followed was as described by Hartman et al. (2) with minor modifications. The sample (1-5 g of oil, depending on P content) was weighed into a PTFE crucible. Sodium hydroxide (2 mL, 50% w/v) and ethanol (5 mL) were added and the liquids were evaporated at 100 C on a hot plate. The crucibles were then heated at 180 C in a forced air oven to completely saponify the sample. The solution was acidified by boiling with 20 mL of 3 N HCl. The organic material was removed by extraction with hexane using separatory funnels. The aqueous residue was made up to 50 mL in a volumetric flask and phosphorus was determined colorimetrically using the molybdenum blue procedure.

Atomic Absorption

The procedure followed was basically as described by Prevot and Gente (3). A sample of oil was diluted 1:1 with methylisobutylketone. Twenty microliters was introduced into the graphite furnace of an atomic absorption spectrophotometer equipped with an electrodeless discharge lamp using argon as the purge gas. The temperature was programmed from 20 to 300 C in 30 sec with a further increase from 300 to 1,300 C requiring 2 min. Atomizing took place at

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2,800 C with regulation by photodiode of 5 sec. The phosphorus absorption was measured at 213.6 nm and phosphate was calculated from a standard curve using lecithin.

Molecular Emission Cavity Analysis (MECA)

The procedure was followed as described by Rogers and Downey (4). A sample of oil (20 μL) was placed in the sample cup of the MECA analyzer and the sample cup was placed in the hydrogen-rich flame. The molecular emission of phosphorus (HPO) was measured at 526 nm. A multiple injection procedure was used for samples with low phosphorus levels. Phosphate was estimated from a standard curve prepared from lecithin.

RESULTS AND DISCUSSION

Using the AOCS Official Method, Laboratories A and C generally agreed within ± 5% for samples with greater than 100 ppm phosphorus and within ± 2 ppm for samples with less than 100 ppm phosphorus (Table I). This agreement is good, considering the reported precision. Laboratory B gave somewhat higher results using the molybdovanadophosphate procedure but this was thought to be possibly due to a calibration error (using Na_2HPO_4 as a standard rather than NaH_2PO_4). Laboratories B and C both claimed excellent agreement between molybdenum blue and molybdovanadophosphate colorimetric procedures and preferred the latter since it is less sensitive to changes in acid concentration. (It does require the use of perchloric acid.)

The main problems with the AOCS Official Method seem to be the long time required to ash the sample (up to 2 days) and the possibility of losing part of the sample in the ignition procedure. Laboratory B reported that ignition of the fumes from the samples on the hot plate significantly reduced the ashing time.

The perchloric acid digestion procedure gave consistently lower results than the AOCS procedure (Table II). The precision of the procedure within a batch was fair (± 5 ppm) but poor reproducibility was found between batches (± 50-100 ppm) with the result that, while single batches gave a good rank order correlation with the AOCS method, individual results do not compare well. The procedure has the advantage of a high throughput, a relatively short analysis time (2 hr) and requires a minimal amount of sample. The procedure has the disadvantages of a lack of accuracy and the use of perchloric acid in the digest which requires special safety precautions. Also, the digests are often dark brown or yellow colored and this might interfere with later colorimetric measurements.

The oxygen bomb procedure (Table II) gave results which were slightly lower than those from the AOCS procedure. The reported precision of ± 4 ppm P is not quite as good as the AOCS (± 2 ppm). This procedure has the advantage of speed (ca. 45 min/determination) and requires only small amounts of sample. The equipment is fairly inexpensive.

The saponification procedure (Table II) gave results which were within about 10% of results from the AOCS procedure except with the crude oils. The low precision might be improved with practice. Sampling problems (handling gummy oils) may be the cause of large differences observed with crude oil samples. The procedure is relatively simple but lengthy and labor intensive (ca. 8 hr). There would seem to be potential for developing a semimicro scale procedure.

Results from the atomic absorption procedure (Table III) were within 10% of the AOCS procedure for samples with more than 100 ppm P and within ± 3 ppm for samples with lower values. The precision of this method has been reported to be ± 5% at the 3 ppm level. (B.F. Teasdale, personal communication). Problems with response to inorganic phosphorus (Teasdale, pers. com.) may make this procedure unsuitable for oils which have been treated with phosphoric acid. Workers in France (Teasdale, pers. com.) have found that the addition of organic lanthanides overcome the problem with inorganic phosphorus. This was not tried in our study.

Results from the MECA procedure (with one exception) were also within 10% of the results from the AOCS Official Method. The precision has been reported to be ± 10% at the 3 ppm level (4). In this study, the MECA did not detect phosphorus in samples with less than 30 ppm although the detection limit with organic phosphorus is 2 ppm. This suggests that the difficulty in detecting inorganic phosphorus is even greater with MECA than with atomic absorption. The MECA procedure was the most rapid of all the procedures tested and required the least sample handling.

Besides the difficulty with inorganic phosphorus (Teas-

TABLE I

Comparison of Results for Analysis of Phosphorus in Canola Oil Using AOCS Official Method Ca 12-55[1]

Oil and type		Phosphorus content (ppm) Laboratory A	Laboratory B[2]	Laboratory C	Mean
Crude	a	834[3]	879 ± 6[4]	820[5]	844
	b	283	363 ± 1	290	312
	c	173	209 ± 4	168	183
Degummed	a	241	300 ± 2	231	257
	b	292	373 ± 2	301	322
	c	361	463 ± 5	378	400
Caustic refined	a	5	9 ± 2	6	7
	b	7	12 ± 4	7	9
	c	22	34 ± 3	25	27
Refined and bleached	a	26	37 ± 2	28	30
	b	18	24 ± 3	20	21
	c	2	2.2 ± 3	2	2

[1] Methods were slightly modified as described in text.

[2] Used the molybdovanadophosphate procedure.

[3] Single determinations.

[4] Mean of 2 (crude and degummed) or 3 determinations.

[5] Mean of 2 determinations. Standard deviation of ± 2.0 ppm reported.

TABLE II

Comparison of Results from Various Digestion-Spectrophotometric Procedures for Determining Phosphorus in Canola Oil

Oil type		Phosphorus content (ppm)			
		Mean AOCS	Perchloric acid[1]	Oxygen bomb[2]	Saponification[3]
Crude	a	844	444	776	1127 ± 242
	b	312	186	279	199 ± 20
	c	183	123	162	117 ± 18
Degummed	a	257	166	223	235 ± 21
	b	322	193	286	281 ± 15
	c	400	222	348	360 ± 28
Caustic refined	a	7	14	7	7 ± 2
	b	9	19	9	8 ± 1
	c	27	17	25	27 ± 3
Refined and bleached	a	30	23	28	15 ± 3
	b	21	13	18	10 ± 1
	c	2	ND	3	2 ± 1

[1] Single determination.

[2] Mean of 2 determinations. Precision of ± 4.3 ppm reported.

[3] Mean of 3 determinations.

TABLE III

Comparison of Results from Two Instrumental Procedures for Determination of Phosphorus in Canola Oil with Results from the AOCS Official Method

Oil type		Phosphorus content (ppm)		
		Mean AOCS	Atomic absorption[1]	Molecular emission cavity analyst[1]
Crude	a	844	816	647
	b	312	304	297
	c	183	162	189
Degummed	a	257	232	248
	b	322	294	290
	c	400	396	365
Caustic refined	a	7	5	ND
	b	9	6	ND
	c	27	22	12
Refined and bleached	a	30	38	ND
	b	21	23	ND
	c	2	2	ND

[1] Mean of 2 determinations.

dale, pers. com.) the MECA procedure does not have the same sensitivity as atomic absorption unless multiple burns are carried out (4). Both instrumental procedures are rapid and use a minimum of sample but require relatively expensive equipment.

In summary, it would appear that of the methods examined for analysis of phosphorus by digestion followed by spectrophotometric phosphate determination, both the oxygen bomb procedure and the saponification procedure give results which are comparable to the AOCS official procedure. The oxygen bomb ashing technique would appear to be the best choice for routine analyses, although both the saponification and perchloric acid digestion procedures might be suitable in special circumstances. Atomic absorption appears to be superior to MECA as an instrumental procedure, although both procedures have some drawbacks, especially when dealing with samples containing inorganic phosphorus.

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