☘Flavor Stability of Soybean Oil Based on Induction Periods for the Formation of Volatile Compounds by Gas Chromatography

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ABSTRACT

Although previous research showed that volatile compounds detected by gas chromatography (GC) correlated well with flavor scores, no instrumental or chemical method has been available to predict flavor stability of vegetable oils reliably. A direct GC method was tested to predict flavor stability of soybean oil by measuring induction periods based on the time required for rapid formation of volatile compounds. By this technique, induction periods of 9, 5 and 0 days were obtained with oils containing a combination of tertiary butylhydroquinone (TBHQ) and citric acid (CA), CA only and no additives, respectively. Addition of methyl silicone to the oils containing CA or CA + TBHQ did not increase their stability. Prominent peaks identified by gas chromatography-mass spectrometry included: pentane, hexanal, 2-heptanal, 2,4-heptadienal, 2-decenal and 2,4-decadienal. Measures of total volatile compounds, pentane and 2,4-decadienal were best related to deteriorative changes. High correlation coefficients were obtained between individual and total volatiles with flavor scores. This study showed that flavor stability of oils can be predicted by determining induction periods based on the formation of volatile compounds.

INTRODUCTION

Sensory evaluation generally is considered to be the ultimate method to measure flavor quality of vegetable oils. Chemical or instrumental procedures such as peroxide values, free fatty acids, color development or gas chromatographic (GC) analysis for volatile compounds are used widely to evaluate oil deterioration. However, all of these methods lack the acuity of the human senses and the ability to integrate perceptions. In the last 20 years, there has been an increase in the use of direct GC methods for volatile compounds as a measure of oil and food quality. Scholz and Ptak (1) developed a direct injection procedure to detect pentane as a marker for rancidity in cottonseed and other vegetable oils. Evans and coworkers (2,3) published direct GC procedures to evaluate edible oil quality by the thermal release of pentane. Later, other researchers, including Jarvi et al. (4) and Dupuy et al. (5), developed simplified, direct GC techniques to analyze for pentane and total volatiles. Williams and Wille (6) as well as Waltking and Zmachinski (7) outlined refinements in the direct GC procedures to improve speed of analysis.

Previous research at this laboratory (8) and by several other groups (7,9-11) reported high correlations of flavor scores of oils with volatile compounds measured by the direct method. However, without knowledge of the history of the oil tested, it is not possible to predict flavor stability on the basis of volatiles analysis. Our objective in this study was to evaluate the relative flavor stability of soybean oil by measuring induction periods, based on the time required for rapid formation of volatile compounds under known storage conditions. A number of additives were tested to generate oils of different quality. The levels of volatile compounds measured by GC were correlated with flavor scores to determine how this technique can be used to predict flavor stability.

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EXPERIMENTAL

Materials

A commercial refined, bleached soybean oil was deodorized in the laboratory (12,13) and treated with different additives (Table I) obtained from commercial sources: citric acid (CA) (J.T. Baker Chemical Co., Phillipsburg, New Jersey); methyl silicone (MS) ("Antifoam A" from Dow Corning Corp., Midland, Michigan) and tertiary butylhydroquinone (TBHQ) (Eastman Chemical Products, Inc., Kingsport, Tennessee). The additives were incorporated in the oils on the cooling side of deodorization as follows: CA, 20% aqueous solution; TBHQ, 10% ethanolic solution and MS, neat. The water and ethanol were removed during the cooling of the oils under vacuum in the final deodorization stage. All oils were held at 0 C until sensory and volatile compounds analyses were carried out.

The oils were aged at 60 C by a modification of the Schaal oven test (14), using 8-oz narrow-mouth clear glass bottles filled 2/3 with oil. It was previously estimated that 4 days of storage at 60 C was equivalent to 4 mo at room temperature (15).

Gas Chromatographic Volatile Analysis

Volatile compounds were measured by adaptations of direct GC analyses previously reported (3,16). A Perkin-Elmer gas chromatograph, Sigma 3B model (Oak Brook, Illinois) was used with a flame ionization detector and a glass column (6 ft × 1/4 in. O.D. × 2 mm i.d.) packed with 8% PolyMPE (polymetaphenoxylene) on 60/80 mesh Tenax GC (Applied Science, Warrenville, Illinois). A 15-μl oil sample was injected directly onto a small plug of silanized glass wool placed inside the inlet liner of the injector. The glass plug was replaced after each sample injection. The column temperature was programmed from 0 to 250 at 5 C/min with a final hold of 10 min; injector temperature was 180 C; detector temperature was 250 C and carrier gas was helium. Although the first major peak did not elute until 100 C, better separation of peaks was obtained at an initial program temperature of 0 C. For quantitative analysis, ethyl acetate was used as an internal standard because it eluted after pentane at 130 C where no other peaks were detected. Peaks were identified by mass spectral analyses with a Kratos MS 30 (Manchester, England) (17) and confirmed by matching GC retention times with those of authentic compounds. Each oil was analyzed for volatiles in duplicate. Headspace analyses of volatiles was done with a Perkin-Elmer headspace analyzer attachment (Model HS 6) to the GC. GC conditions for headspace measurement were the same as those for the direct analysis method, except that 1 g of oil was heated to 180 C in the analyzer for 16 min before GC programming.

Peroxide Value Analysis

Induction periods based on the time required for rapid increases in peroxide values were determined by aging 50 g of oil in 150-ml glass beaker (in duplicate) at 60 C in a

forced-air draft oven. One-gram aliquots of oil were removed periodically for peroxide value determination by AOCS Method Cd 9-53 (18).

Sensory Evaluation

A 15-member trained, experienced oil panel evaluated the oils for odor and flavor on the basis of a 1-10 scoring scale, with 10 as bland and 1 as strong. Panelists tasted 10 ml of oil heated to 50 C in a 50-ml clear glass (Pyrex) beaker covered with a watch glass. Testers recorded their ratings by using Votomatic Vote Recorders (Computer Election System, Berkeley, California). Perforated computer cards, one each for odor and flavor, were placed separately in the recorder. Testers recorded their data by punching perforations in the card corresponding to various scores and description intensities. Cards were read by a mechanical card reader, and data were calculated by computer.

Statistical Analyses

Oils were presented to testers in orders based on balanced incomplete block design (19). Each panelist tested three oils per sitting, and 18 scores were obtained for each sample. Analysis of variance and least significant different (LSD) were calculated for all flavor scores. Correlation coefficients were obtained between flavor scores and integrator counts for both total volatiles and individual GC peaks. Means of overall flavor scores were calculated, as well as weighted averages of individual flavor description intensities (20).

RESULTS AND DISCUSSION

The stability of the oils was estimated by following the formation of volatile compounds during various storage times at 60 C. The effects of additives on induction periods of aged soybean oil are shown in Figure 1. Induction period is defined as the time at which the slope for volatile compound formation changes sharply. The induction curves plotted in Figure 1 from total volatiles showed three rates of oil deterioration. As expected, the soybean oil control with n additives had the shortest induction period of 1 day. The addition of CA or CA + MS extended the stability of the oil to a 5-day induction period. The most effective additive combination, TBHQ + CA, increased the oil stability to an induction period of 9 days. The addition of MS to oils containing CA or CA + TBHQ had no effect on SBO stability under these experimental conditions. This result confirmed previous work (21).

The stability of oils often has been measured on the basis of induction periods from either peroxide values or oxygen uptake (22-24). However, these measures of oxidation did not correlate well with flavor deterioration (25,26). For comparison with total volatiles, the stability of the soybean oil samples was monitored by the induction period based on peroxide values. The number of days for induction were longer for peroxide values than for GC volatile compounds (Fig. 2). The samples with either CA or CA + MS had induction periods of 5 days as measured by volatiles and 6 days by peroxide values. The oils treated with TBHQ + CA had induction periods of 9 days based on volatiles and 11 days based on peroxide values. Correlation coefficients calculated for flavor scores vs. induction periods by peroxide values and by total volatiles were -0.56 and -0.96, respectively. Induction period measurements by the peroxide method showed no changes until 5 days for the oil treated with CA + TBHQ (Fig. 2). In contrast, an increase in volatile compounds was detected with these samples after 1 day of storage.

Flavor evaluations of the fresh and aged SBO samples showed distinctive patterns of flavor deterioration (Table I).

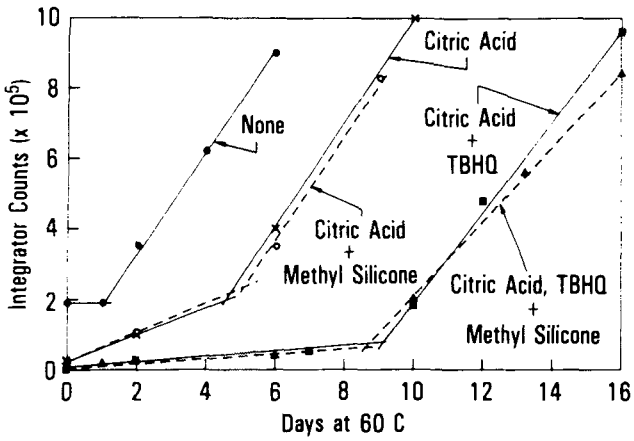

FIG. 1. Induction periods for SBO by direct GC analysis of total volatiles. Samples were: (—●—) control (no additives); (—X—) 100 ppm citric acid (CA); (- - -0- - -) 100 ppm CA + 5 ppm dimethylpolysilicone (MS); (—■—) 100 ppm CA + 200 ppm tertiary butylhydroquinone (TBHQ) and (- -△- -) 100 ppm CA + 5 ppm MS + 200 ppm TBHQ.

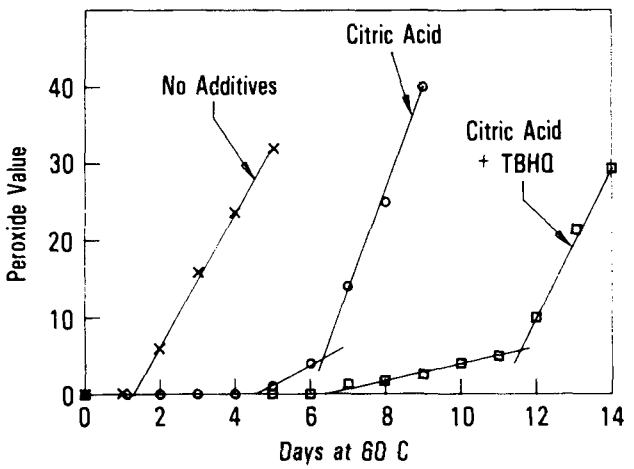

FIG. 2. Induction periods for soybean oil based on peroxide values.

TABLE I

Flavor Scores[a] of Initial and Aged Soybean Oils[b]

Additives	Days at 60 C				
	0	2	4	8	16
None	6.4	5.1	4.2	3.4	–
0.01% Citric acid	7.2	7.3	7.2	6.0	5.1
0.01% Citric acid + 5 ppm methyl silicone	8.1	–	–	6.4	4.6
0.01% Citric acid + 0.02% TBHQ	7.8	–	–	7.1	6.4
0.01% Citric acid + 0.02% TBHQ + 5 ppm methyl silicone	8.3	–	–	6.9	6.2

[a]Scores based on 1-10 scale with 10 as bland and 1 as strong.
[b]Least significant difference (LSD) = 0.7.

Soybean oil without additives (control) showed lower scores initially than the other treated oils because of incipient oxidative deterioration between the time of deodorization and later flavor evaluations. The flavor score of the control oil with no additives declined steadily between the initial and the 4-day storage evaluations. In the presence of CA, the oil maintained a high flavor score after aging for 4 days, followed by significant drops in scores after 8 and 16 days. This flavor deterioration coincided with the induc-

tion periods for formation of volatiles (Fig. 1). The time of the actual break in flavor score could not be determined, because the oils were not tasted as often as the volatiles were measured. As with volatile analyses, the addition of CA + MS had no effect on flavor score compared with CA only. In the presence of TBHQ, the scores of the oils remained near 7.0 after storage for 8 days but dropped significantly after 16 days. The addition of MS to the oil containing TBHQ also coincided with induction periods based on volatiles analyses. If a score of 6 is considered as the end point for acceptable quality, then the times necessary to reach this score are in good agreement with the induction periods based on volatiles analyses.

Predominant peaks were identified by gas chromatography-mass spectrometry as due to pentane, hexanal, 2-heptenal, 2,4-heptadienal, 2-nonenal, 2-decenal and 2,4-decadienal, in order of elution. These peaks appeared consistently in all chromatograms and differed only in relative heights. Correlation coefficients between the flavor scores and integrator counts of GC peak areas were calculated to be: -0.96, total volatiles; -0.93, pentane; -0.63, hexanal; -0.83, 2-heptenal; -0.92, *cis*-2,*trans*-4-heptadienal; -0.68, *trans*-2, *trans*-4-heptadienal; -0.63, 2-nonenal; -0.71, 2-decenal; -0.96, *cis*-2,*trans*-4-decadienal and -0.94, *trans*-2,*trans*-4-decadienal. These volatile profiles obtained by direct injection-packed column GC for soybean oil aged at 60 C are similar to those reported by St. Angelo et al. (27) for soybean oil exposed to light. The correlation coefficients in our study for total volatiles, pentane and 2,4-decadienal agree with those calculated by Jackson and Giacherio (10) for soybean oils aged at room temperature under normal fluorescent lighting and by St. Angelo et al. (27) for the same oils exposed to high-intensity fluorescent light.

Markers other than total volatiles are of interest because large peaks such as pentane might contribute little to the flavor of an oil, whereas a small peak might have a low flavor threshold and, therefore, have a large influence on flavor (20,26,28). To investigate the relative importance of different volatiles, the induction period for pentane formation was compared with that of hexanal and 2,4-decadienal. Pentane had a significant influence on total volatiles because its induction period was 11 days, compared to 9 days for total volatiles (Fig. 2). Although hydrocarbons have high threshold values for flavor detection (29), they are sensitive markers because they are formed initially during oxidative aging of oils (30,31). Hexanal gave an induction period of 14 days, compared to 12 days for 2,4-decadienal and 11 days for pentane. These results are consistent with those of Horvat et al. (30) and Selke et al. (31), who observed hydrocarbons forming before aldehydes in vegetable oils.

Earlier work on volatile analyses as an instrumental method of flavor measurement (32-34) was based on headspace analysis. The increased use of headspace techniques prompted us to compare induction periods based on headspace with those based on direct analysis (Fig. 3). A sample of soybean oil containing CA + TBHQ was evaluated for total volatiles by these two techniques. Induction periods were 9 days and 14 days, respectively, by direct and headspace analysis. Therefore, changes in flavor deterioration are detected at an earlier point by the direct GC method than by the headspace technique. The smaller amount of oil (15 μl) used in the direct GC method than in the headspace method (1 g) may cause more severe oxidative decomposition of the sample and result in shorter induction periods. Further work is needed, however, to correlate flavor scores with headspace volatile analyses.

This study shows that the flavor stability of soybean oil can be estimated readily and reliably by direct GC analysis.

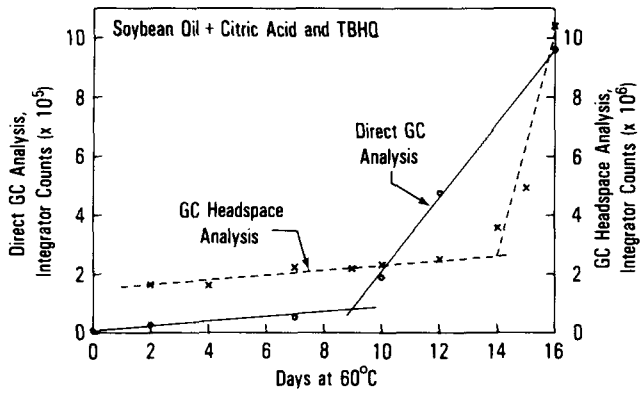

FIG. 3. Induction periods by direct GC and GC headspace analysis of total volatiles.

Total volatiles, pentane and 2,4-decadienal correlated well with flavor scores. Induction periods based on formation of volatile compounds can, therefore, be used to predict relative differences in flavor stability of soybean oil without always having to rely on expensive monitoring of flavor by sensory panels.

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L.A. Parrott conducted gas chromatographic analysis, R.E. England did mass spectral analysis and W.F. Kwolek and W.J. Bailey did the statistical analysis.

REFERENCES

1. Scholz, R.G., and L.R. Ptak, JAOCS 43:596 (1966).
2. Evans, C.D., JAOCS 44:366A (1967).
3. Evans, C.D.; G.R. List, R.L. Hoffman and H.A. Moser, JAOCS 46:501 (1969).
4. Jarvi, P.K.; G.D. Lee, D.R. Erickson and E.A. Butkus, JAOCS 48:121 (1971).
5. Dupuy, H.P.; S.P. Fore and L.A. Goldblatt, JAOCS 48:121 (1971).
6. Williams, J.L., and J.H. Wille, JAOCS 53:634 (1976).
7. Waltking, A.E., and H. Zmachinski, JAOCS 54:454 (1977).
8. Warner, K.; C.D. Evans, G.R. List, H.P. Dupuy, J.I. Wadsworth and G.E. Goheen, JAOCS 55:252 (1978).
9. Dupuy, H.P.; E.T. Rayner and J.I. Wadsworth, JAOCS 53:628 (1976).
10. Jackson, H.W., and D.J. Giacherio, JAOCS 54:458 (1977).
11. Morrison, W.H.; B.G. Lyon and J.A. Robertson, JAOCS 58:23 (1981).
12. Schwab, A.W., and H.J. Dutton, JAOCS 25:57 (1948).
13. Mounts, T.L.; K. Warner, G.R. List, J.P. Friedrich and S. Koritala, JAOCS 55:345 (1978).
14. Joyner, N.T., and J.E. McIntyre, Oil and Soap 15:184 (1938).
15. Evans, C.D.; G.R. List, H.A. Moser and J.C. Cowan, JAOCS 50:218 (1973).
16. Dupuy, H.P.; S.P. Fore and L.A. Goldblatt, JAOCS 50:340 (1973).
17. Frankel, E.N.; W.E. Neff and E. Selke, Lipids 18:353 (1983).
18. Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1, 2nd ed., AOCS, Champaign, IL, 1964. (Revised to 1969). Method Cd 8-53.
19. Cochran, W.G., and G.M. Cox, Experimental Designs, 2nd ed., John Wiley & Sons Inc., New York, N.Y., 1957.
20. Warner, K.; T.L. Mounts, J.J. Rackis and W.J. Wolf, Cereal Chem. 60:102 (1983).
21. Sims, R.J.; J.A. Fioriti and M.J. Kanuk, Lipids 8:337 (1973).
22. Sherwin, E.R., JAOCS 55:809 (1978).
23. Odumosu, O.T.; J. Sinha and B.J.F. Hudson, J. Sci. Food Agric. 30:515 (1979).
24. Jackson, H.W., JAOCS 58:227 (1981).
25. Fritsch, C.W., and J.A. Gale, JAOCS 54:225 (1977).
26. Frankel, E.N., Prog. Lipid Res. 22:1 (1983).
27. St. Angelo, A.J.; M.G. Legendre and H.P. Dupuy, Autoxidation in Food and Biological Systems, M.G. Simic and M. Karel, ed., Plenum Press, New York, 1980, p. 171.
28. Patton, S., and D.V. Josephson, Food Res. 22:316 (1957).

29. Stahl, W.H., ed., Compilation of Odor and Taste Threshold Values Data, American Society of Testing and Materials, Philadelphia, PA (1973).
30. Horvat, R.J.; W.G. Lane, H. Ng and A.D. Shepard, Nature 203:523 (1964).
31. Selke, E.; H.A. Moser and W.K. Rohwedder, JAOCS 47:393 (1970).
32. Nawar, W.W., and I.S. Fagerson, Food Technol. 16:107 (1962).
33. Warner, K.; C.D. Evans, G.R. List, B.K. Boundy and W.F. Kwolek, J. Food Sci. 39:761 (1974).
34. Fioriti, J.A.; M.J. Kanuk and R.J. Sims, JAOCS 51:219 (1974).

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Simultaneous Determination of Moisture and Oil Content In Oilseeds by Pulsed Nuclear Magnetic Resonance

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ABSTRACT

Pulsed nuclear magnetic resonance technique using Carr-Purcell-Meiboom-Gill (CPMG) sequence has been used for simultaneous determination of moisture and oil content in rapeseed-mustard. This method involves sampling the free induction decay (FID) following 90° pulse in the CPMG sequence and resolving the trace of the amplitude of the CPMG echo signals into exponentially decaying liquid components of oilseeds. The data show that water in oilseeds generally exists in 2 phases and the relatively slow decaying component disappears around moisture content of 7% and below. The moisture and oil content have been determined by the method for 34 samples of 5 different varieties of seeds at varying moisture levels (~3% to 22%). The measured moisture and oil content have been compared with the values obtained by the oven drying method and earlier known FID method of pulsed nuclear magnetic resonance (NMR) respectively, and the agreement is fairly good for rapid estimation with standard deviation of 0.70% for oil content and 0.99% for moisture content. This is a rapid and nondestructive method for determination of both moisture and oil content without weighing and drying the seeds and also seems suitable for other matrix samples.

INTRODUCTION

Proton NMR techniques have been used extensively for determination and improvement of oil content in oilseeds (1-4). The continuous wave wide-line and pulse NMR techniques, being rapid and non-destructive methods for determination of oil content, have acquired great importance in plant breeding programs. The measurement of oil content by these methods in seeds containing moisture exceeding 5% requires drying of samples in order to eliminate interference by moisture signals. In addition, samples have to be weighed, which reduces the speed of the method (5). The free induction decay (FID) method of pulse techniques has been developed further to estimate oil content without weighing and drying the samples, but this method requires that moisture content should not change significantly from one sample to another (6). The different pulse techniques, namely Free Induction Decay (FID), spin echo (SE) and Carr-Purcell-Meiboom-Gill (CPMG) sequence (7,8), for estimation of oil content, have been compared. It is reported that relative oil content estimated by the Carr-Purcell-Meiboom-Gill (CPMG) sequence is not much influenced even at 20% moisture content (9). However, the CPMG method as performed previously required dry weight of the samples to estimate oil content. The pulsed NMR methods also have been used for moisture determination in barley, corn, wheat (10) and paddy (11) seeds.

Both oil and water content in olive husk have been determined simultaneously by solid to liquid ratio and SE decay curve of the sample (5). In the present paper, the feasibility of the CPMG method of the pulsed NMR technique for simultaneous determination of moisture and oil content in oilseeds is established. This rapid and nondestructive method does not require either weighing or drying of the sample and has significant applications in postharvest storage, germ-plasm evaluation, physiological studies, the food industry and marketing and plant-breeding programs.

EXPERIMENTAL METHODS

A pulsed NMR spectrometer operating at 20 MHz, Bruker Minispec pc 20 was used for all measurements. The 90° pulse width and the receiver dead time were about 4 μsec and 9.5 μsec (from middle of 90° pulse) respectively. The r.f. coil used provided a uniform field up to the sample height of 25 mm in a sample tube of 12 mm diameter. The sample height was restricted to 20 mm, and about 1.5 g of seeds were used for each analysis. The L/L + S EDM from Bruker was installed to operate the instrument, but the required pulse sequence was entered manually. The measured CPMG echo amplitudes were read out and recorded manually for analysis. The diode detection mode was used with 1 MHz band width and the probe temperature was around 40 C.

Seed samples of 5 rapeseed-mustard varieties—Pusa-Bold, Varuna, BS58, RLM198 and DYS-1—were hydrated by storing the seeds in a desiccator over water for about a week. The moisture content in hydrated seeds was decreased slowly by natural air drying and storing the seeds over calcium chloride in a desiccator at room temperature. By this process we were able to dry the seeds down to approximately 3% moisture content. Final drying was done in an oven at 105 C. Oil percentage in the seeds dried at 105 C was determined by the FID method of pulsed NMR technique (4).

For each sample, a CPMG pulse sequence with 180° pulse spacing of 0.1 msec was used. The signal was sampled at even echoes leading to measured data points every 0.2 msec. One hundred measurements were averaged for each sample to improve S/N ratio. The repetition time for individual pulse sequences was 2.0 sec, leading to a total measurement time of about 200 sec. In addition, the FID following the 90° pulse in CPMG sequence also was sampled just after the dead time of the receiver. The representative FID's were stored on a Nicolet Explorer III digital