한작지(Korean J. Crop Sci.), 51(S): 283~291(2006)

Non Destructive Fast Determination of Fatty Acid Composition by Near Infrared Reflectance Spectroscopy in Sesame

Churl-Whan Kang*†, Dong-Hwi Kim**, Sung-Woo Lee**, Ki-Jong Kim**, Kyu-Chae Cho***, and Kang-Bo Shim*

**Yeongnam Agricultural Research Intitute, NICS, RDA, Milyang 627-803, Korea*
***National Institute of Crop Science, RDA, Suwon 441-100, Korea*
****Dooree Tech. Inc., 303 Sogang Bld. Changan 1 Dong Tongdaemungu, Seoul 130-101, Korea*

ABSTRACT To investigate seed non destructive and fast determination technique utilizing near infrared reflectance spectroscopy (NIRs) for screening ultra high oleic (C18:1) and linoleic (C18:2) fatty acid content sesame varieties among genetic resources and lines of pedigree generations of cross and mutation breeding were carried out in National Institute of Crop Science (NICS). 150 among 378 landraces and introduced cultivars were released to analyse fatty acids by NIRs and gas chromatography (GC). Average content of each fatty acid was 9.64% in palmitic acid (C16:0), 4.73% in stearic acid (C18:0), 42.26% in oleic acid and 43.38% in linoleic acid by GC. The content range of each fatty acid was from 7.29 to 12.27% in palmitic, 6.49% from 2.39 to 8.88% in stearic, 12.59% of wider range compared to that of stearic and palmitic from 37.36 to 49.95% in oleic and of the widest from 30.60 to 47.40% in linoleic acid.

Spectrums analyzed by NIRs were distributed from 400 to 2,500 nm wavelengths and varietal distribution of fatty acids were appeared as regular distribution. Varietal differences of oleic acid content good for food processing and human health by NIRs was 14.08% of which 1.49% wider range than that of GC from 38.31 to 52.39%. Varietal differences of linoleic acid content by NIRs was 16.41% of which 0.39% narrower range than that of GC from 30.60 to 47.01%. Varietal differences of oleic and linoleic acid content in NIRs analysis were appeared relatively similar inclination compared with those of GC.

Partial least square regression (PLSR) among multiple variant regression (MVR) in NIRs calibration statistics was carried out in spectrum characteristics on the wavelength from 700 to 2,500 nm with oleic and linoleic acids. Correlation coefficient of root square (RSQ) in oleic acid content was 0.724 of which 72.4 percent of sample varieties among all distributed in the range of 0.570 percent of standard error when calibrated (SEC) which were considerably acceptable in statistic confidence significantly for analysis between NIRs and GC. Standard error of cross validation (SECV) of oleic acid was 0.725 of which distributed in the range of 0.725 percent standard error among the samples of mother population between analyzed value by NIRs analysis and analyzed value by GC. RSQ of linoleic acid content was 0.735 of which 73.5 percent of sample varieties among all distributed in the range of 0.643 percent of SEC. SECV of linoleic acid was 0.711 of which distributed in the range of 0.711 percent standard error among the samples of mother population between NIRs analysis and GC analysis. Consequently, adoption NIR analysis for fatty acids of oleic and linoleic instead that of GC was recognized statistically significant between NIRs and GC analysis through not only majority of samples distributed in the range of negligible SEC but also SECV.

For enlarging and increasing statistic significance of NIRs analysis, wider range of fatty acids contented sesame germplasm should be kept on releasing additionally for increasing correlation coefficient of RSQ and reducing SEC and SECV in the future.

Keywords : sesame, fatty acid, NIRs

Sesame is the third major food crop in Korea with average of 53,000 ha (1998) following rice and soybean. Sesame had been transferred to Korean penninsula around 2,000 years ago through China (1,000BC) along the "Silk Road" of Arabian merchants trade route of Tibet, India, Pakistan, Afghanistan, Iran, Iraq, Igypt, Ethiopia and Sudan. Ethiopia has been considered as a world origin of sesame, because a lot of wild species of sesame have been found in these area. Sesame have been acknowledged to start cultivation in these area since 3,000 BC. Sesame has been favored historically by the people of Korea as an essential material of seasonings for side dishes on daily meals of

†Corresponding author: (Phone) +82-55-350-1236 (E-mail) cwkang@rda.go.kr

housewives kitchen by oil, raw grain itself, crushed after slight roasting, traditional cookies made of sesame grains attached on sorghum gluten painted on popped glutinous rice, glutinous sesame cracker and rice soup made of ground black seedcoat color of sesame. Sesame traditionally for Korean patients has been used for a health recovery food, noodles into sesame soup, boiled chicken and soup mixed into sesame soup, kneading wheat flour mixed with honey and wine roasted into sesame oil, final touch of two or three sesame oil drops for special flavour added into various seasoning vegetable side dishes, a spoonful of sesame oil added into rice food mixed with seasoned vegetables or seasonings, etc. Sesame is a long history crop in Korea especially much favored in the housewives kitchen for a seasoning crops on the various side dishes of vegetables, meats, rice soup, etc. Sesame grain itself, ground one and oil mechanically extracted through frying in local market or in big oil corporations which occupying more half of national sesame oil consumption are rather old fashioned usage but recently popularizing hamburger breads, fast foods spraying frying dehulled grains on the surface and crackers, etc. are the new ones.

"Screening for High Quality" is to be considered a tool for a qualitative improvement breeding in general. "Screening for High Quality" in The Korea Republic should be the most urgent and important breeding goal in major and minor crops cultivated. Due to high cost of local crops production, competition has been so hard with introduced one from countries of advanced technology of fully automated in vast land area and underdeveloped plentiful, low wages manpower. Selling high quality crops by good price in local market is absolutely important for farmers to compete with imported ones.

Consumption of sesame in Korea has been growing up but downward trend in local production caused by increasing importation since historic wide acreage year of 1987 with 94,000 ha. Price of local sesame is higher than that of imported one because of it's high quality and good taste. People do not hesitate paying money for better taste and quality of local sesame. Development good quality high yielding new varieties has a very important role of local sesame production promising to farmers high income through good market price. High oleic acids variety is preferable for salad oil to prevent concretion and harmonious balance of oil with linoleic acid. High linoleic acid of essential vitamin F variety is preferable for high blood pressure removing LDL cholesterol.

Qualitative characteristics of local sesame in the market for purchasing farmers and consuming housewives are depending upon their eyes and hand touching feels for identifying between local and imported ones. The criteria of deciding quality are as follows : (1) Seedcoat color : consumers are generally favour pure whiteness with traditional beliefs of better tastes and quality than those of brown ones, dark brown or gray while especial healthy food effects of black ones. (2) Taste : pure white Korean landraces is selling as the best taste and quality in cusumer market. The aromatic compounds are accumulated in inner seed coats mainly composed of various phenol compounds and have been mostly favored from the people of Korea but disliked from Europeans traditionally by the reason of the same aromatic flavor of which Korean favored. Commercialized sesame seeds in Europe and America has been used under the condition of dehulled seeds due to traditional unfavorable custom to the aromatic flavor of sesame. (3) Seed size : small seed size has been generally favored because of the small seeds of the Korean local cultivars. (4) Maturity : well matured grain has oval shape, pure whiteness or black, shine and cool hand touch feeling on the seed coat surface in general. (5) Constitutional compounds : people are not well acknowledged not only importance of profitable components for human health but also varietal differences between locally produced and imported ones, especially among young generations. Probably people are well awared of and confided to some good healthy compounds of sesame specially produced inland.

More than ninety-five percent of oil is composed of fatty acids. Major fatty acids of sesame are saturated palmitic (C16:0), stearic (C18:0), unsaturated oleic (C18:1) and linoleic acid (C18:2). Ten percent around of palmitic and stearic acid are unprofitable for health but ninety percent of oleic and linoleic acid are profitable (Bae *et al.*, 1996).

Individual plants (lines) among segregation pedigree generation shall be able to be analyzed by NIRs through nondestructive and fast analysis. This analysis could not only get data of relative fatty acid content statistically significant with ease and fast but save seeds from analysis for planting next year by non-destructive (Moon *et al.*, 1994).

As an efficient tool breeding for qualitative improvement, utilizing NIR is to be considered as the most effective and fast way to reach to the goal of breeding for developing high quality and yielding cultivars up to now. Fatty acid composition analysis by NIRs is the first step of sesame qualitative component improvement breeding in NICS (National Institute of Crop Science).

Finally, through releasing thousands and hundred thousands of segregating pedigree plants (lines) and germplasms toward NIRs, selecting ultra high oleic and linoleic fatty acids varieties will greatly contribute to develop high quality and yielding commercialized varieties successfully.

MATERIALS AND METHODS

NICS (National Institute of Crop Science) has been carrying out research projects of breeding for improving qualitative components with sesame. The project started with fatty acid composition analysis using NIRs. Hundreds of sesame germplasm were grown for analysis through seed multiplication in experimental field of NICS since 1997. 150 among 378 cultivars were analyzed fatty acids by GC (VARIAN 600 USA) and NIRs (NIR Systems Model 6500 USA). Calibration statistics were carried out through the computer systems of NIR with analyzed values by NIRs and GC.

GC analysis was carried out under methylation by sodium methoxide, 200℃ and 220℃ of glass column filled with 15% diethylene glycol succinate and injector. FID detector and helium gas as carrier by flow rate of 20 mm/min. were used. NIRs analysis was carried out under range from 400 to 2500 nm spectrum wavelengths using seed filled spinning sample cup and obtained continuous spectrums averaged through 32 times repeated beam scanning injections utilizing spinning module which were measured area of spinning sample cup. 150 cultivars among 378 were analyzed unsaturated oleic and linoleic fatty acids by GC and NIRs, and calibrated statistically by the computer systems of NIR. (Han *et al.*, 1996).

RESULTS AND DISCUSSION

Distribution of fatty acids among 150 sesame cultivars of introduced and Korean landraces is shown in the Table 1. Average content of fatty acids was 9.64% in palmitic with the range from 7.29 to 12.27%, 4.73% in stearic with the range from 2.39 to 8.88%, 42.26% in oleic with wider range from 37.36 to 49.95% and 43.38% in linoleic with the widest range from 30.60 to 47.40 %.

Varietal differences of minimum to maximum value on each fatty acid among 150 cultivars was 4.98% in palmitic, 6.49% in stearic, 12.59% in oleic and 16.80% in linoleic. Good quality of unsaturated fatty acids in oleic and linoleic were appeared to be wider range than worse quality of saturated palmitic and stearic. Wider range of oleic and linoleic acid promise high probability for quality improvement breeding.

Histogram distribution of palmitic acid by GC analysis is shown in Figure 1. It showed regular distribution with the range of 4.50% from 7.54 to 12.04%. The majority of sam-

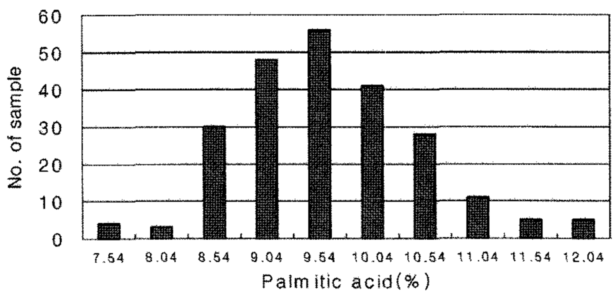

Fig. 1. Histogram distribution of palmitic acid by GC analysis in sesame.

Table 1. Distribution of fatty acids analyzed by gas chromatography in sesame.

No. varieties Released	Fatty acid composition (%)			
	C16:0	C18:0	C18:1	C18:2
150 (Max)	12.27	8.88	49.95	47.40
150 (Min)	7.29	2.39	37.36	30.60
Difference (Max-Min)	4.98	6.49	12.59	16.80
150 (Mean)	9.64	4.73	42.26	43.38

ples were distributed on 9.54% with 56 sample varieties.

Histogram distribution of stearic acid by GC analysis is shown in Figure 2. It showed regular distribution but inclined to lower content on the range from 2.72 to 8.57%. The majority content of samples were distributed on 4.67% with 114 sample varieties.

Histogram distribution of oleic acid by GC analysis is shown in Figure 3. It showed regular distribution inclined to lower content in wider range from 37.99 to 49.33%. The majority content of samples were distributed on 43.03% with 65 sample varieties. The highest variety of oleic acid content in GC analysis was Korean landrace-50 with 49.69%.

Histogram distribution of linoleic acid by GC analysis is shown in Figure 4. It showed regular distribution inclined to higher content reversely in stearic and oleic acid with the widest range of 15.12% from 31.44 to 46.56% among fatty acids. The majority content of samples were distributed on 43.20% with 88 sample varieties. The highest variety of linoleic acid was UCR-182 introduced from USA with 47.40%.

Histogram distribution of oleic acid by NIRs analysis is shown in Figure 5. It showed regular distribution inclined

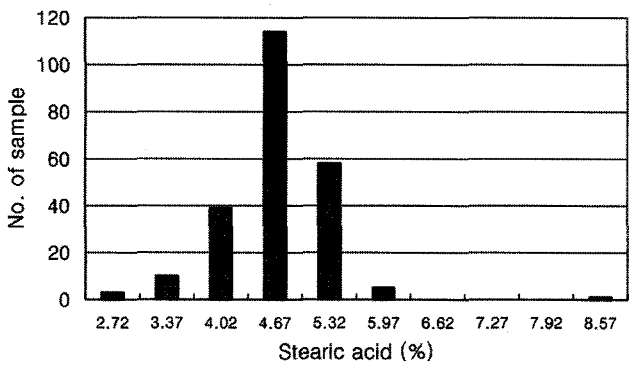

Fig. 2. Histogram distribution of stearic fatty acid by GC analysis in sesame.

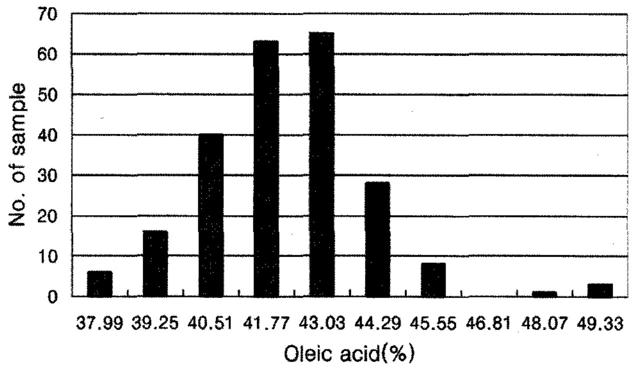

Fig. 3. Histogram distribution of oleic fatty acid by GC analysis in sesame.

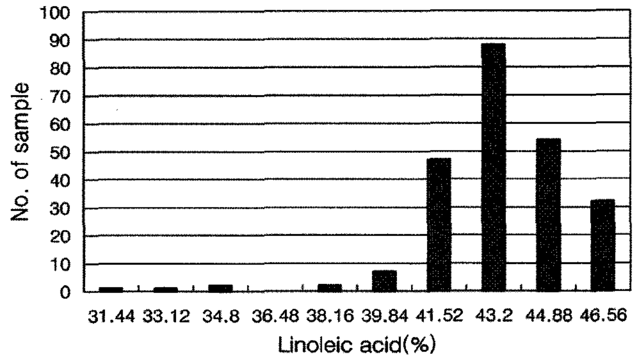

Fig. 4. Histogram distribution of linoleic fatty acid by GC analysis in sesame.

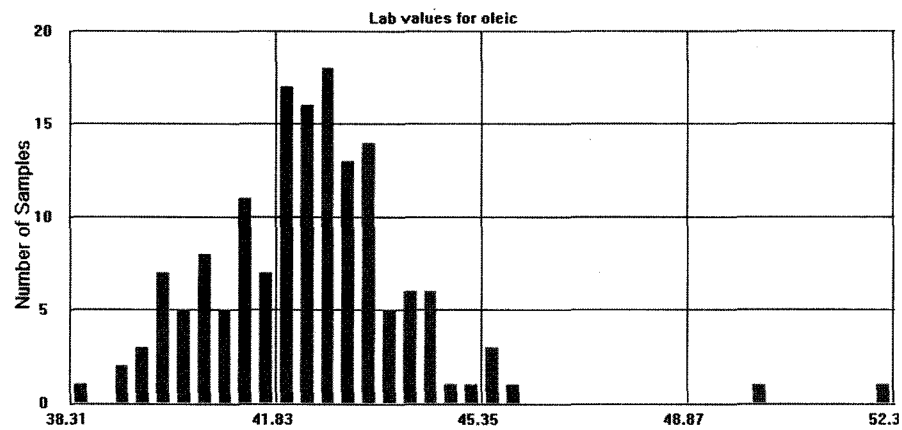

Fig. 5. Histogram distribution of oleic fatty acid by NIRsanalysis in sesame.

to lower content such as in GC with the range of 14.08% from 38.31 to 52.39%. The majority content of samples were distributed on 42.88% with 18 sample varieties. NIRs analysis of oleic acid was appeared 2.74% wider in range but 0.15% lower in majority sample content than that of GC.

Histogram distribution of linoleic acid by NIRs analysis is shown in Figure 6. It showed regular distribution inclined to higher content such as GC analysis in the range of 16.41% from 30.60 to 47.01%. The majority content of samples were distributed on 43.73% with the 18 sample varieties. NIRs analysis of linoleic acid was appeared 1.29% wider in range and 0.53% higher in majority sample content than that of GC.

Unstable histogram distribution with relatively narrow range of oleic and linoleic acids content analyzed by NIRs and GC may happen to cause decrease correlation coefficient of root square (RSQ) and increase standard error of calibration (SEC) for composing calibration statistics. Wider range of fatty acids content in sample varieties with replicated analyses are the most important matter for composing calibration statistics using NIRs.

Spectrum characteristics from 400 to 2,500 nm wavelengths of sesame varieties were analyzed by NIRs such as Figure 7. Changes of colour in the wavelengths from 400 to 700 nm was caused by differences of seedcoat colour on sample varieties. Peak of C-H combination band on the wavelength of 2,200 nm was ruined seriously by spectrum characteristics but 1,700 nm peak spectrum of the first over-

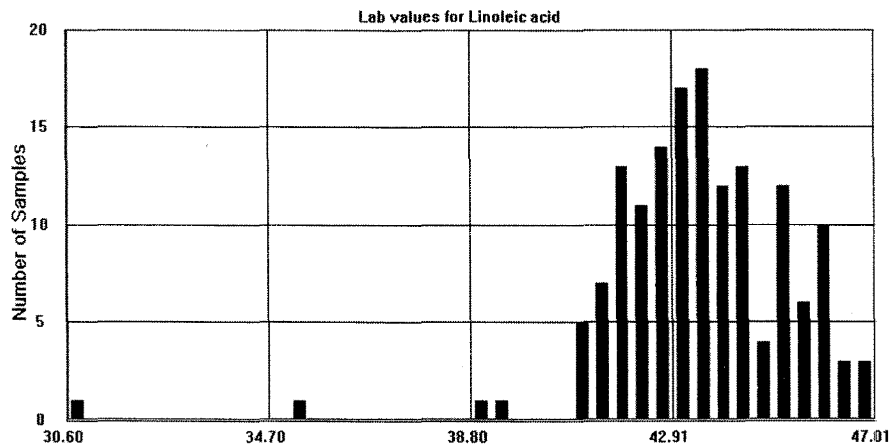

Fig. 6. Histogram distribution of linoleic fatty acid by NIRsanalysis in sesame.

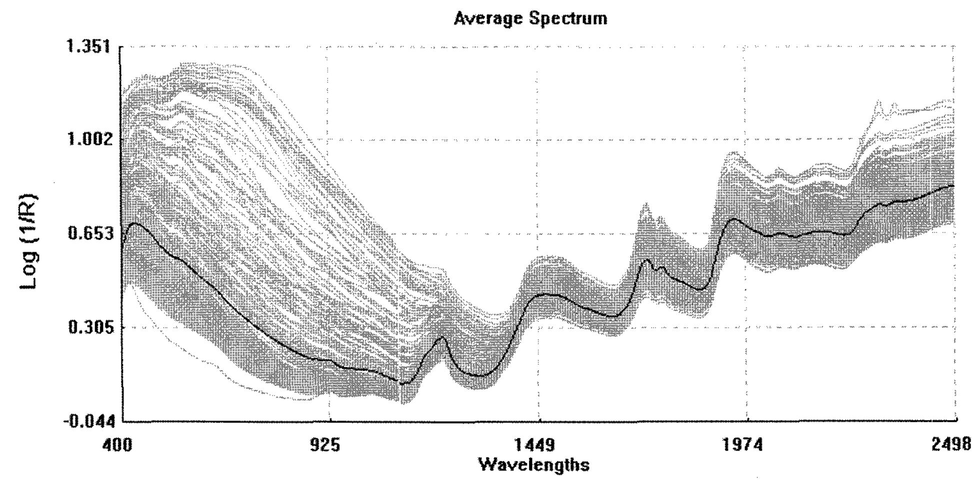

Fig. 7. Spectrum characteristics from 400 to 2,500 nm wavelengths by NIRs analysis in sesame.

tone band of C-H was clear. Peak of water absorbable strong combination band was distributed on the wavelength of 1,940 nm, 1,460 nm in peak of first overtone band and slight N-H band was found on 2,060 nm. Peaks on those spectrum characteristics of NIRs were seriously duplicated and C-H band related with oleic and linoleic were too. Those spectrum characteristics should be considered correlationships between neighboring in peaks not by independently.

Principal component analysis (PCA) was carried out to compose sample population structure utilizing spectrums drawing above. Numbers of basic factors were necessary to compose PCA due to similarity of sample characteristics and drew three axis graph such as Figure 8. Most of all samples were located in central part but outside in a few. It was supposed to be further differences of seed shape, seedcoat colour and it's kinds and quantity of chemical components compared to those of central major sample varieties.

Analysis of mother population by PCA could get not only confident calibration statistics through identifying sample ranges but enlarging range of statistical confidence of unknown samples and calibration ; furthermore, get identifying variables of varieties, specific characteristics and origins.

150 sample varieties were analyzed for composing calibration statistics of oleic and linoleic acids by GC and NIRs. Derived spectrums by NIRs were different by degrees of grain shape and seedcoat colour not much by differences of fatty acid content. Scatter correction and derivatives were modified such as Figure 9 to support correctness and minimize error through excluding spectrums of seedcoat colour, etc. for composing calibration statistics. Those process could increase not only correctness but confidence in calibration through minimizing differences

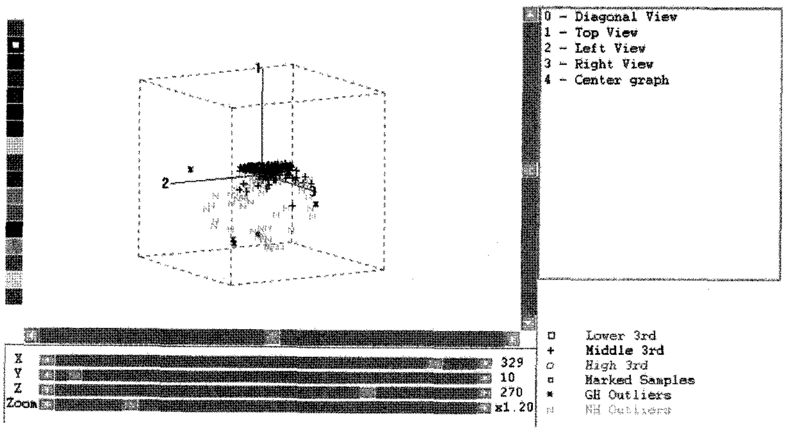

Fig. 8. Displaying sample population by three axis for principal components analysis (PCA) in sesame.

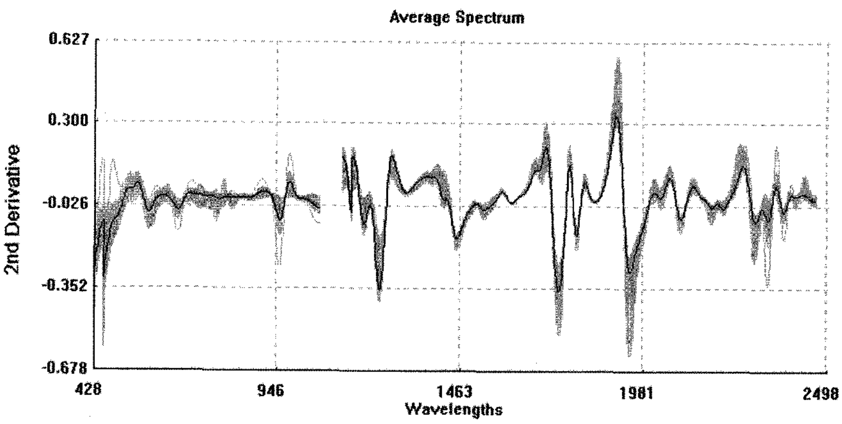

Fig. 9. Scatter correction and spectrum on second derivatives by NIRs analysis in sesame.

of base lines caused by grains and spectrum overlapping.

Analysis of partial least square regression (PLSR) among multiple variant regression (MVR) utilizing derived spectrums from 700 to 2,500 nm wavelengths was carried out such as Table 2.

Correlation coefficient of root square (RSQ) was 0.724 of which 72.4 percent sample varieties among all distributed in the range of 0.570 percent of standard error in calibration (SEC) and the residual 27.6 percent sample varieties distributed out the range of 0.570 percent. SEC 0.570 means 0.570 percent standard error of oleic acid content may occurred in calibration statistics. Standard error of cross validation (SECV) derived from unknown seed samples analysis by NIRs adopting principal component analysis (PCA) was 0.725 of which recognized statistically significant 0.725 percent standard error of oleic acid may occurred between NIR and GC. Those results of not only majority of 70 percent more samples among all mother population distributed in the range of negligible below 0.6 percent SEC but all mother population distributed under 0.8 percent SECV in analysis between NIRs and GC were accepted as significant statistically. Calibration curve of oleic acid by the regression statistics was drawn in Figure 10.

PLSR of linoleic acid was carried out such as Table 3,

Table 2. Regression statistics of GC and NIRs analysis of oleic acid content in sesame varieties.

Modified PLS Regression Statistics		2-12-99			
Input File		SS98C.CAL	REP File		None
Validation File		None	Equation File		ss98.eqa
Math Treatment		1,4,4,1	Number of Variables		221
Scatter Corr.		SNV and Detrend	Downweight Outliers		No
Constituent		Oleic	Number of Samples		118
Mean	42.193	Range	39.61 - 44.53	Std Dev.	1.085
	SEC	RSQ	F	SECV	1-VR
1	0.924	0.274	45.21	0.942	0.258
2	0.820	0.429	32.35	1.000	0.165
3	0.772	0.493	15.66	0.916	0.299
4	0.723	0.556	17.09	0.841	0.409
5	0.697	0.587	9.59	0.840	0.411
6	0.657	0.634	15.13	0.825	0.432
7	0.623	0.670	13.45	0.793	0.475
8	0.587	0.708	14.92	0.738	0.545
9	0.570	0.724	7.57	0.725	0.561

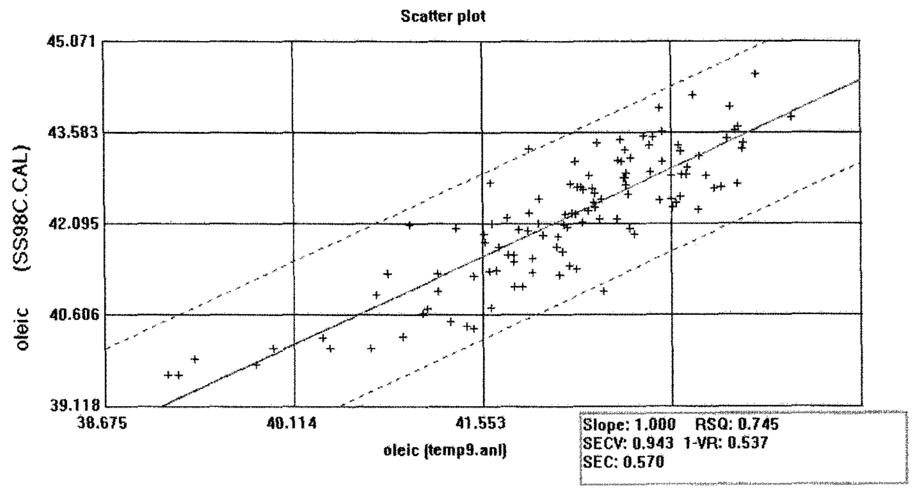

Fig. 10. Calibration curve of GC and NIRs analysis on oleic fatty acid content in sesame varieties.

Table 3. Regression statistics of GC and NIRs analysis on linoleic fatty acid content in sesame varieties.

Modified PLS Regression Statistics		2-12-99			
Input File		SS98C.CAL	REP File		None
Validation File		None	Equation File		ss98.eqa
Math Treatment		1,4,4,1	Number of Samples		144
Scatter Corr.		SNV and Detrend	Downweight Outliers		No
Constituent		Linoleic	Number of Samples		144
Mean	43.525	Range	41.31 - 46.72	Std Dev.	1.248
	SEC	RSQ	F	SECV	1-VR
1	0.978	0.386	72.01	0.979	0.378
2	0.792	0.598	59.95	0.868	0.512
3	0.685	0.699	38.13	0.754	0.632
4	0.643	0.735	15.99	0.711	0.672

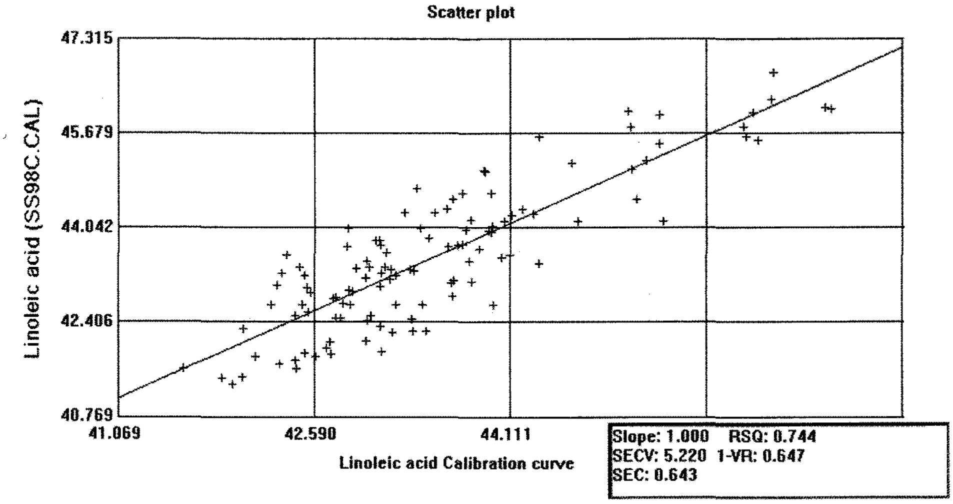

Fig. 11. Calibration curve of GC and NIRs analysis on linoleic fatty acid content in sesame varieties.

and calibration curve was drawn such as Figure 11. Correlation coefficient of RSQ was 0.735 of which 73.5 percent among all distributed in the range of 0.643 percent. SEC and the residual 26.5 percent distributed out the range of 0.643 percent. SECV 0.711 was recognized statistically significant by 0.711 percent standard error of linoleic acid in analysis between NIRs and GC.

Consequently, analysis of oleic and linoleic acid content using NIRs instead of GC were recognized statistically significant for qualitative improvement breeding through seed non-destructive and fast analysis with segregating pedigree lines (plants) and germplasms in sesame. However, it is still strongly requested to release additional cultivars of wider range of oleic and linoleic fatty acid content to NIRs calibration analysis in order to not only increase statistic confidence of correlation coefficient of RSQ but reduce SEC and SECV significantly on sesame in the future.

REFERENCES

Bae, Y. M., S. I. Cho, and J. G. Chun. 1996. Measurement of fat content in potatochips by near-infrared spectroscopy. Korean J. Food Sci. Technol. 28(5) : 916-921.

Cha, I. S., J. H. Kim, H. W. Kim, H. C. Kim, Y. K. Lee, K. M. Park, and M. Y. Yoo. 1996. Measurement of mayonnaise salt content by near-infrared reflectance spectroscopy. Korean J. Food Sci. Technol. 28(1) : 40-43.

Cho, S. Y., S. G. Choi, and C. Rhee. 1994. Determination of degree of retrogradation of cooked rice by near-infrared reflectance spectroscopy. Korean J. Food Sci. Technol. 26(5) : 579-584.

Han, C. S. and M. Y. Natsuga. 1996. Development of a constituent prediction model of domestic rice using near infrared reflectance analyzer. I. Constituent prediction model of brown and milled rice. Korean J. Agric. Mach. 21(2) : 198-210.

Kim, J. M., B. K. Min, and C. H. Choi. 1997. Determination of rice milling ratio by visible near-infrared spectroscopy. Korean J. Agric. Mach. 22(3) : 333-342.

Moon, S. S., K. H. Lee, and R. K. Cho. 1994. Application of near infrared reflectance spectroscopy in quality evaluation of domestic rice. Korean J. Food Sci. Technol. 26(6) : 718-725.