Studies on the Lipids and Fatty acid compositions of mature grain of three different varieties of corn (Zea mays L.)

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Abstract — Changes in lipid content and fatty acid compositions of mature grain of three different varieties of corn have been studied. The lipids were extracted from the mature grain by using a solvent mixture of chloroform, methanol and water (65:35:4) and fractionated into lipid classes by a combined technique of column and thin layer chromatography. In all three varieties the synthesis of lipids and fatty acid compositions were most active between 15 and 45 days after pollination. Triglycerides (TG) constituted 15-41 % of the total lipids at 15 days and increased to 70-90% at 90 days after pollination. The saturated fatty acids of the TG fraction of all varieties decreased with the maturity of the grain. But the unsaturated fatty acids specially oleic and linoleic acids were gradually increased with the maturity of grain and it attained the highest percentage after 90 days of pollination. The synthesis of polar lipids represented 44.7-70.9% and 4-15.4% at 15 and 90 days respectively. It is interesting to note that polar lipids decreased rapidly with the maturity of the grain after 45 days. The physico-chemical characteristics of the oil of three different varieties were observed. The iodine value (130.0) and the percentage of the unsaponifiable matter (1.15) of the Sadaf variety were found to be much more higher than those of other varieties. No significant changes of other characteristics among the varieties were observed.

Index Terms — Corn, pollination, lipids, fatty acids, kernel and mature grain.

1 INTRODUCTION

THE germ of corn is a good source of polyunsaturated edible oil. The oil is semidrying and is used for salad and cooking purposes, soap and in the baking industry. Because of its high linoleic acid content, the oil may be regarded as a blood cholesterol reducing agent (Vaughan *et al* 1970). The refined oil has good keeping quality and is used in pharmacy as a carrier for vitamins (Anon 1976).

In the recent years, the cultivation of corn has been increased tremendously in Bangladesh especially in the Northern region and the yield is also very satisfactory. So, there is an ample scope of setting up of corn based industries in our country. As corn is an exceptional crop which has more diversified uses than those of other major crops like rice, wheat etc, it can play a significant role in the agronomy of Bangladesh by minimizing the shortage of edible oil. Moreover, it is very encouraging to note that the kernel after extraction of oil is a cheap source of carbohydrate, protein, vitamin and minerals for which many protein and vitamin enriched items may be prepared (Kent N.L. 1966) and as a result the dependence upon rice and wheat will be reduced to a great extent. Hence, it is essential to identify the optimum period when corn seed is most actively synthesizing the lipids and fatty acids. So, the present investigation was an attempt to that direction.

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2. Materials and Methods

The corn was grown in the adjoining field of the BCSIR Laboratories, Rajshahi during the period February to April 2007. Grain of three different varieties named Suvra, Barnali and Sadaf were collected at certain *intervals* after the dates of pollination. The husks and silks were removed manually from the immature corn in the field. The ears were then taken to the laboratory and kept in a cool and dry place. The kernels were removed intact from the ears with a dissecting knife. The kernel weight and moisture were determined by weighing 100 kernels before and after drying in a vacuum oven at 60 ^{0}C for 24 hr. The moisture content in the fresh kernel was determined

by IUPAC method (Anon 1979)

The soft and immature grain collected before 60 days of pollination were ground in a glass mortar and the hard grain collected after 60 days of pollination were ground in a grinder machine. The lipids were extracted by homogenizing the grain in a mixture of chloroform, methanol and water (65:35:4). The solvent containing lipids were filtered and the lipids were recovered from the filtrate by evaporating the solvent using a rotary vacuum evaporator. The physical and chemical characteristics of the extracted lipids of each varieties were determined by standard AOCS methods (Anon 1980).

2.1 Fractionation of lipid classes

The total lipid extracts were fractionated into three major lipid classes by silicic acid column chromatograph (Rouser et al 1967). The silicic acid (E. Msark Darmstadt, Germany 70-230 mesh) was washed with water and methanol to remove fine particles and impurities. It was then activated at 120^{0}C overnight and again for 1 hour immediately before the column was prepared. For each column 25 gm silicic acid was washed with 250 ml of chloroform-methanol (7 :1 v/v), 120 ml chloroform-methanol (15:1% v/v) and 160 ml chloroform. A slurry of 25 gm of silicic acid in chloroform was poured into column (2.2 cm id). After the column was washed with 100 ml diethyl ether and 325 ml 4% diethyl ether in petroleum ether (b.p. 60-70^{0}C), 150 mg of the total lipids were dissolved in 5 ml chloroform and quantitatively transferred to the column following (Rahman et al 1997).

The neutral lipid was eluted by 80 ml of chloroform, glycolipids by 200 ml of acetone and phospholipids by 175 ml of methanol (Robertson et al 1978). The elution was controlled with a flow rate of 1.5-2 ml/ min. The elution of each fraction was monitored by microslide thin layer chromatography (TLC) to ensure uniformity of separation of each lipid class during silicic acid chromatography and the eluted solvents were collected in a weighed flask. The fractions thus obtained were evaporated in a rotary vacuum evaporator and were dried under reduced pressure before being weighed. The percentages of these fractions were determined by gravimetric method.

2.2 Separation of fatty acids by GLC

The fatty acid composition of triglyceride (TG) fraction of each variety was analysed as their methyl esters which was prepared by the boron triflouride methanol method following Gofur et al (1993). The analysis was carried out by a GCD Pye Unicam gas chromatography equipped with a flame ionization detector. Nitrogen carrier gas was used as a flow rate of 30 ml/min. Fatty acids esters were separated on a 1.8m×2 mm id. glass column packed with 6% BDS (Butanediol Succinate Polyesters) on solid support Anakoram ABS 100/120 mesh. Analysis was carried out at isothermal column temperature of 190^{0}C, injector and detector temperature for all GLC analysis was 200^{0}C. Gas chromatographic peaks were identified by comparison with standard methyl esters with respect to retention times, by plotting the log of retention times against equivalent carbon length (ECL). The peak areas were determined by multiplying peak height by peak width at half height. The percentage of each peak was calculated as the percentage of the total area of all the peaks.

Pancreatic lipase hydrolysis of the triglycerides was performed by a semi-micro technique following the procedure of Luddy et al (1964). About 9 mg pancreatic was added to 50 mg of weighed triglycerides in a screw cap vial. Then 1 ml of 1M trisbuffer (trihydroxy methyl amino methane, pH adjusted to 8.0) 0.1 ml 22% calcium chloride solution and 0.25 ml of 0.1% bile salt solution were added. The preheating and shaking were carried out at 40^{0}C. At the end of reaction the content of the vial was acidified with 0.5 ml of 6N HCl and immediately transferred to a small separating funnel and extracted with diethyl ether. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated.

The extract of the hydrolysate was separated on a 20 cm x 20 cm plate coated with a layer of 0.5 mm thick of silica gel G with a solvent system petroleum ether – diethyl ether – acetic acid (70:30:1) . The developed plate was sprayed with 2,7-dichlorofluorescein to locate the 2-monoglyceride by comparison with standard reference samples. The band was scrapped off and extracted with chloroform. The eluent was evaporated to dryness under a stream of nitrogen and converted to methyl esters by the method described earlier. The fatty acid of 2-

monoglyceride were analysed by gas liquid chromatography.

3 Results and Discussion

The developing corn kernels of three different varieties have been studied to evaluate the changes in weight of kernels, oil content and fatty acid composition of the lipid classes. The physico-chemical characteristics of the extracted oil were determined and the results were shown in Table-1. The results are in good agreement with the results of Uddin et al (2007). From the result it was observed that the main characteristics like fatty acid compositions varied from 1.4-1.8, unsaponifiable matter from 0.9-1.15%, peroxide value from 0.5-0.7%, saponification value from 73-75 and iodine value from 107.5-130 depending upon the variety. Changes in weights and oil contents of kernels were depicted in Table-2. Based upon the results in Table-2 it was found that both wet and dry weights of kernels were rapidly increased from 15 to 45 days after pollination in all three varieties and after 45 days their increment were slower. In between 60 to 90 days the rate of increase in weight of kernels began to level off or somewhat decrease. Oil content of all three varieties were gradually increased up to 90 days after pollination. But in case of Sadaf, the oil content was somewhat decreased after 45 days and it was level off at 90 days. The percentage of oil content of Barnali was the highest at 90 days (5.0%) and that of Sadaf was the lowest (3.9%) when the grain was fully matured (Table–2).

Fractionation of the total lipid extracts on silicic acid column chromatography revealed that as the grain matured, the overall properties of the lipid classes changed (Table-3). It was observed that the sterol esters and hydrocarbons in all varieties were highest in the initial stage but decreased gradually up to the maturity of the grain. It was observed that the increase of triglycerides in between 15 to 45 days was very rapid in all three varieties and they were gradually increased with the maturity of the kernels (Table-3). The percentage of polar lipid was highest in the initial stage for three varieties and it was remarkable to note that the decrease of polar lipids was very sharp in between 15 to 45 days. After that the decrease was very slower up to the maturity (Table-3). It was also observed that the percentages of hydrocarbons and sterol esters, fatty acids, sterols and partial glycerides were decreased gradually with the maturity (Table-3). In all three varieties at 15 days after pollination the polar lipid fractions represented approximately 70% of the total lipids, but as the storage lipids, the triglycerides were synthesized, the proportion of polar lipids declined rapidly with the maturity of grain. At maturity 75.1% of the total lipids of Sadaf were triglycerides, Suvra were 81.2% and Barnali were 90.5%. The fatty acid compositions of triglyceride fractions were analysed by gas liquid chromatography and the results were presented in Table-4. It was observed that the percentage of palmitic, stearic and linolenic acids fell and those of oleic and linoleic acids increased through out the whole period of maturity. After 45 days the proportions of fatty acids in the triglyceride remained nearly constant. At maturity the percentage of linoleic acid (18:2) was the highest in all three varieties (Table-4).

Based upon the above results it may be concluded that corn oil is very rich in linoleic and oleic acid for which it may be considered as a potential source of unsaturated fatty acid. Considering the lipid content, percentage of triglycerides and unsaturated fatty acids, Barnali variety may be recommended as the source of a good edible oil.

Table 1: Physical and chemical characteristics of corn oil from differentvarieties of dried ground corn after 90 days.

Variety	Sp. gravity at 20^0C	Refractive index at 15^0C	FFA (%)	Saponification value	Unsaponifiable matter (%)	Iodine value	Peroxide value (%)	Oil content (%)
Suvra	0.916	1.471	1.4	75	0.9	115.5	0.5	4.6
Barnali	0.919	1.472	1.7	73	0.9	107.5	0.7	5.0
Sadaf	0.921	1.471	1.5	73	1.15	130.0	0.5	3.5

Mean result of three experiments

Table 2: Changes in weight and oil content of different varieties of corn.

Strain	DAP	100 kernels		Dry wt (%)
		Wet wt. in gm.	Dry wt in gm	
Suvra	15	15.5	2.4	2.8
	45	30.2	17.5	3.9
	60	32.1	22.5	4.2
	90	32.2	22.5	4.5
Barnali	15	12.5	7.5	2.5
	45	28.5	15.5	3.9
	60	29.6	17.2	4.5
	90	29.5	16.9	5.0
Sadaf	15	10.5	2.3	3.5
	45	25.6	18.8	4.5
	60	30.5	20.3	3.9
	90	29.9	19.5	3.9

DAP = days after pollination (wt./100 kernels)

Table 3: Fractionation of Lipid classes of different varieties of corn oil.

Strains	DAP	Neutral lipids					Polar lipids (%)
		Hydrocarbons-sterol esters (%)	Triglycerides (%)	Free fatty acids (%)	Sterols (%)	Partial glycerides (%)	
Suvra	15	4.5	28.9	1.0	5.9	4.2	55.5
	45	2.8	71.5	0.7	4.9	4.5	15.6
	60	2.8	77.5	0.7	4.5	3.5	11.0
	90	2.5	81.2	0.5	4.0	3.1	8.7
Barnali	15	4.3	41.0	0.5	5.0	4.5	44.7
	45	2.1	83.9	0.5	3.1	4.4	6.0
	60	1.5	87.0	0.4	2.9	3.2	5.0
	90	1.0	90.5	0.4	1.5	2.5	4.1
Sadaf	15	4.9	15.3	0.9	4.5	3.5	70.9
	45	3.5	72.5	0.7	3.9	2.5	16.5
	60	3.4	73.1	0.5	3.5	2.4	17.1
	90	3.0	75.1	0.5	3.9	2.1	15.4

DAP = days after pollination

Table 4: Fatty acid composition (Triglyceride) of differentvarieties of corn oil.

Strain	DAP	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)
Suvra	15	13.0	1.6	36.5	46.0	2.5
	45	12.5	1.5	37.0	47.6	1.3
	60	12.0	1.5	37.5	48.0	0.5
	90	11.5	1.2	38.3	48.5	0.5

Barnali	15	13.5	1.8	25.5	52.5	6.7
	45	12.8	1.5	28.6	53.7	3.4
	60	11.5	1.5	29.5	54.2	3.3
	90	11.0	1.2	32.1	55.5	1.2
Sadaf	15	19.5	1.9	20.1	49.5	9.0
	45	18.0	1.5	21.5	50.2	8.8
	60	17.1	1.5	25.3	51.5	4.6
	90	15.5	1.2	29.2	51.5	2.6

DAP = days after pollination

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