Altering Fatty Acids of Sunflower to Improve Oil Stability

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Introduction

Traditional high-linoleic sunflower oil has been viewed as a healthful vegetable oil with desirable flavor and has been considered a premium oil in world markets because of its high percentage of polyunsaturated fatty acids. Its popularity in the world for salad oil, cooking oil, or for margarine production was based on its oil composition and the absence of cholesterol. However, to stabilize sunflower oil for shipping or storage, hydrogenation is used to change the oil from a liquid to a solid form. This hydrogenation process creates trans fatty acids that are of concern for health conscience people. A new sunflower oil has been developed which will achieve excellent oxidative stability in the liquid form under storage and shipping conditions and also is more stable than traditional sunflower oil under frying conditions. To achieve this improvement, the oil needs an oleic acid concentration of 55 to 65%, a linoleic acid level of 20 to 28%, and a saturated acid level less than 9%.

Commercial samples of traditional sunflower oil and mid-oleic sunflower were stored for 2, 4, 6, and 8 days at 60 C and compared with the original oils at 0 days storage (Warner, 2000). The peroxide value (mEq/Kg) was measured to determine the oxidative stability of the oil. The equivalent real time storage would be one month, two months, three months, and four months for the 2, 4, 6, and 8 days at 60 C, respectively. At all storage times, the traditional oil was significantly lower in stability than the mid-oleic oil with peroxide values at 8 days being 26 mEq/Kg for the traditional oil versus only 17 mEq/Kg for the mid-oleic oil. Confection sunflower kernels were roasted in traditional and mid-oleic sunflower oil and then the kernels were stored for 28 weeks (Robertson et al., 1988). The free fatty acid (FFA) level was measured as an indication of oxidative stability of the stored product. Both kernel products were essentially equal in FAA after 12 weeks of storage. At 16 weeks of storage, the kernels roasted in traditional oil began to rise significantly in FFA and after 28 weeks the differences were highly significant, with the slope of the FFA curve indicating serious deterioration of the kernels roasted in traditional oil. The kernels roasted in oleic sunflower oil were relatively stable and did not become rancid in taste after 28 weeks (6 months). Yodice (1990) also measured stability of traditional sunflower oil versus mid-oleic sunflower oil using an active oxygen method (AOM) where oxygen is forced through a sample of oil at 97.8 C and the peroxide value of the oil is measured as a indication of stability. After 21 days of storage the AOM value of traditional sunflower was 12

whereas the AOM value of mid-oleic sunflower oil was 23. Thus, the mid-oleic oil was more stable, taking nearly twice as long to produce hydroperoxides which, along with their breakdown products have been implicated in promoting cancer.

Inheritance of oleic acid concentration in sunflower

Plants of the traditional oleic inbred line, HA 89, were hand-emasculated and pollinated with a selection from the high-oleic open-pollinated cultivar Pervenets (PI 483077). HA 89 had approximately 11% oleic acid whereas the Pervenets selection had 84% oleic acid. The F_1 (Pervenets/HA 89) crossed seeds had 52.0% oleic acid concentration. These F_1 seeds were planted in the field at Fargo, ND, and were self-pollinated to produce F_2 seed. Single seeds were used in the fatty acid analysis, and segregation ratios were tested by a Chi-square goodness of fit and heterogeneity analysis.

Analysis of the F_2 population showed trimodal distributions for oleic acid concentration. A mid-oleic class was clearly evident, ranging in oleic concentration from 48 to 72%. The high oleic class ranged from 82 to 92%, whereas the low oleic class was similar to HA 89 and ranged from 11 to 18%. The number of seeds in the mid-oleic class was too large to support a single, dominant gene theory. However, it did identify a major dominant gene with partially dominant gene action, designated *Ol*. This gene produced seed with oleic concentration levels of 50 to 75%. A second gene, designated *Ml*, appears to modify the oleic content. When in the recessive form, *mlml*, and combined with the gene *Ol*, oleic levels in seed were 82% or higher. HA 89 appeared to possess dominant alleles of the modifier gene. A theoretical genotype *Ol__Ml__* would produce mid-oleic concentration, whereas the *Ol__mlml* genotypes would produce high oleic concentration. Food processors have recognized that the flavor of food fried in mid-oleic sunflower oil (50-75% oleic acid) has superior flavor compared with traditional sunflower oil or very high oleic sunflower oil (80% or higher) (Campbell et al., 2000). Therefore, breeding for a mid-oleic concentration would be a valid objective for researchers of sunflower in developing countries to produce a sunflower oil with improved oil stability.

Breeding Strategies for Producing an Open-Pollinated Mid-Oleic Sunflower Variety

Two methods are suggested for breeding an open-pollinated mid-oleic sunflower variety. These are the Recurrent Half-sib Selection method and the Stratified Mass Selection method (Miller, 1987). Each will be described in detail.

Recurrent Half-sib Selection. The initial population to begin the recurrent half-sib selection method can consist of a combination of high-performing open-pollinated varieties, inbred lines, plant introductions obtained from a germplasm bank, or a composite of lines from synthetics or composites developed in breeding programs. Key to the initial population would be a source of lines with the

genotype *Ol__Ml__* or *Ol__mlml*. This will allow the cycle 0 (C_0) population to have a relatively high frequency of genotypes with the mid-oleic or high-oleic trait. Once the initial genotypes have been selected, crosses are initiated to produce a random-mated population. Random mating can be accomplished by hand-emasculating plants of each line, and pollinating them with pollen gathered randomly and equally from all other lines or genotypes. Two generations of random matings generally are used to produce the C_0 population.

The C_0 population is planted and grown in isolation at least 1 km from any other sunflower. Individual S_0 plants are selected from the population based on plant characteristics such as height, stem diameter, lodging resistance, head inclination, neck length, resistance to prevalent diseases, and maturity. A population of 400 to 600 plants is recommended as a minimum number to be available for selection purposes. The seed on each selected plant must be protected from bird depredation. At maturity, seed is harvested from each plant by threshing and is then cleaned. Forty to fifty seeds are selected at random from each plant harvested and tested with a refractometer to determine if the *Ol* gene is present. All plants with 50 to 60% oleic are selected. The half-sib seed from the selected plants is used to grow a test plot the following generation with two replications. The half-sib families are evaluated for flowering date, maturity date, height, head inclination, neck length, head diameter, seed yield, seed weight, and if possible, oil percentage. The half-sib families also may be evaluated in disease nurseries separate from the yield nursery. The top 15 to 20% of the S_0plants are selected based on the superior performance of their half-sib progeny. Remnant half-sib seed of the selected S_0 plants is planted in the next generation and random mated to form the C_1 population. Similarly, S_0 plants are selected and tested as described above from the C_1 population. At the end of four cycles of selection, the C_4 population can be tested as an open-pollinated experimental variety and grown in yield trials with competing varieties to determine performance in yield and other critical characteristics and tested for oleic composition by refractometer. If the oleic composition is over 50% and the yield is acceptable, the population can be increased in isolation and released to farmers as a new variety.

An alternative to the recurrent half-sib selection method is the S_1 line progeny test method. The only difference between the two methods is that the S_0 plants are bagged prior to pollination to produce self-pollinated seed. If some degree of self-fertility is required in the open-pollinated variety due to few insect pollinators in the growing or production area, then the S_1 line progeny test method would be preferred. After self-pollination, S_1 seed from the selected plants is grown the following generation in a replicated yield test and evaluations are the same as described above.

Stratified Mass Selection. The stratified mass selection method (Gardner, 1961) is initiated with an initial population the same as described above. There are two types of stratified mass selection: phenotypic selection and family selection.

Phenotypic selection refers to identification of superior S_0 plants in the field, harvesting seed only from those superior plants. Seeds are planted in several grids, with an equal number of plants selected within each grid to equalize environmental or field conditions. A population of 800 to 1000 plants is recommended as a minimum number. No control of pollen is made. The plants are selected for height, maturity, stem diameter, lodging resistance, head inclination, neck length, yield/head, and any disease that is apparent. Since the plants are not bagged, each selected plant must be protected from bird depredation. After seed is harvested from each plant, the oleic percentage is determined by refractometer. All seed from plants with oleic concentrations of 50% or above are bulked, with an equal amount of seed from each plant added to the bulk. The bulked seed is planted the following generation to formulate the C_1generation. After four generations of selection, the bulked plants from the C_4 are tested as an experimental open-pollinated variety in yield trials with competing varieties to determine performance in yield and other critical characteristics and tested for oleic composition by refractometer. If the oleic composition is over 50% and the yield is acceptable, the population can be increased in isolation and released to farmers as a new variety.

Family selection involves selection of individual S_0 plants and subsequent classification of those plants for certain characteristics of interest. For example, one set of plants may be selected for longer season environments and another set may be selected for short season environments. Each plant is harvested and its seed is placed in a bulk with other plants of similar classification. After four cycles of selection the various bulks are planted in isolation for cross pollination and increased for planting as experimental open-pollinated varieties in yield trials.

Refractometer use in determining oleic %

Hand-held refractometers are very inexpensive when compared to the cost of a gas chromatography instrument. Two models have been utilized by the USDA project in Fargo. The Bausch & Lomb model with a refractive index of 1.464 to 1.478 can be used to determine high oleic (1.464 refractive index), mid-oleic (1.4699 to 1.4705), and low-oleic (1.475) seed. The Atago N-4E with a Brix % ranging from 45 to 82% can be used to determine high oleic (71.2), mid-oleic (71.8 to 72.6), and low-oleic (73.2).

Approximately 40 to 50 seeds are compressed to produce three to four drops of oil to place on the hand-held refractometer. A Carver cylinder has been used at the USDA project, with a hydraulic laboratory press used to compress the seed inside the cylinder. The cylinder and surface of the refractometer are cleaned by using hexane solvent in an aerated environment and with people operating the equipment wearing rubber gloves.

The refractometer has been determined to be accurate only to $\forall$ 5% oleic concentration. However, this accuracy has been effective in early selection

procedures to select those genotypes with the *Ol* gene. Therefore, for projects designed to develop open-pollinated varieties with mid-oleic concentrations, the utilization of the reciprocal half-sib selection and mass selection breeding methods using a refractometer would be effective for selection and economical regarding cost for breeding programs.

References

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