EXTRACTION OF ANTHOCYANINS FROM SUNFLOWER HULLS

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RECOVERY OF ANTHOCYANINS FROM SUNFLOWER HULLS

The controversy about synthetic food dyes has led to a renewed interest in natural food colors. Synthetic dyes such as FD + C Red 2 and FD + C Violet #1 have been banned by the FDA (11). With the demise of FD + C Red 2, blue-red coloration has been difficult to attain. There is considerable pressure on the food industry to move away from synthetic dyes (perceived as dangerous by the public) toward natural pigments, especially those pigments providing blue-red coloration (10,11). Many fruits and plant extracts have been successfully used in foods and beverages as a source of "natural" colors. As compared to certified dyes, some of the natural colors have limitations, including less stability than desired. However, techniques are now available to improve color stability. Also, the FDA regulations are beginning to expand the uses of natural colors (1).

Several sources of natural blue-red pigment are used as food additives. Betanins, the major pigments produced by red beets (*Beta vulgaris* L.), are now used widely as a source of blue-red colorant. The main problem with pigments derived from beets is that yield is low: 30 to 100 milligrams of pigment per 100 grams raw beet (10,15). Anthocyanins derived from grape (*Vitis vinifera* spp.) skins (pomace) have been used to fortify the coloration of wines and other food products, but the pigment yield is relatively low, 85 milligrams pigment per 100 grams wet pomace (6). Cranberry (*Vaccinium macrocarpon* AIT.) pomace provides a useful source of anthocyanin pigment, but yield is very low: 15 milligrams pigment per 100 grams wet cranberry pomace (17).

Brummett (2) found that anthocyanins were completely absent from oilseed sunflower (*Helianthus annuus* L.) (black hulls.) However, several sunflower researchers have reported that purple-hulled sunflower genotypes contain anthocyanins within the hypodermal layer of the hull (9,12). The Indians of Southwest United States have extracted pigments from purple-hulled genotypes to dye blankets and other woolen goods for centuries (8). Leclercq (5) reported that anthocyanins is dominant to absence of pigment. Velkor, cited in Stoenescu (12), reported that a second complementary gene loci (homozygous recessive) causes a plant with the pigmentation allele to produce anthocyanins in vegetative plant parts only; hulls contain no anthocyanins. In recent reviews on breeding and genetics of sunflower no negative associations between anthocyanins and economically important characteristics such as yield and oil concentration have been reported (3,12).

The purpose of this study was to extract anthocyanins from purple-hulled sunflower genotypes to determine if such pigments were qualitatively and quantitatively competitive with other sources of natural (blue-red) pigments for food products.

MATERIALS AND METHODS

Achenes from six purple-hulled sunflower genotypes (Nabhan, Hopi, Navaho, Neagra de Cluj, Nain Noir, and Mars) and hybrid 894 were dehulled manually. The former three purple genotypes were grown at Fargo, North Dakota, in 1981 and the latter three purple genotypes were collected from Indian land races in Southwest United States between 1972 and 1979. Since the amount of achenes available from each genotype differed, the amount of hulls used in the extraction process varied from 0.25 to 5.0 g. Anthocyanins were extracted by solvent (95 percent ethanol:1.5N HCL, 85:15) from the hulls by a blending and percolation process described by Fuleki and Francis (4). No extinction coefficient for sunflower anthocyanins has been determined, but the mean coefficient determined by Fuleki and Francis (4) for cranberry anthocyanins was used to determine total concentration of anthocyanins.

The absorbance spectrum for the pigment extracts of each of the six purple-hulled genotypes was determined with a Bausch and Lomb Spectronic 20 spectrophotometer. Small aliquots of the extracted pigment were diluted with the extracting solvent to yield similar pigment concentration for all varieties.

Thin-layer chromatography was performed using glass plates coated with a 250 micron layer of silica gel G. Forestol, a solvent described by Vaccari et al. (12) which contains acetic acid, concentrated hydrochloric acid, and water (30:3:10) was used. R_f values were calculated as the ratio of anthocyanin movement in centimeters to the solvent front movement in centimeters $\times$ 100.

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A two-step extraction was performed to determine the amount of anthocyanins that can be extracted by simple diffusion from intact sunflower schenes. Three replications of 25 whole achenes of a purple-hulled genotype (Neagra de Cluj) were stirred slowly in 70 milliliters of the extracting solution for 3 hours. The amount of anthocyanins in solution was calculated using the aforementioned method. The hulls of the 25 schenes then were removed and the remaining hull anthocyanins extracted and quantified.

The quantity of other acidified ethanol soluble material that was being extracted in the extraction process was determined. Three 1 gram samples of hulls from Neagra de Cluj were dried to 100 percent dry matter, weighed, and the anthocyanins extracted and quantified. Hulls were recovered and dried to 100 percent dry matter. The amount of dry matter lost by the hulls was calculated and the concentration of anthocyanins and other materials in the extract was determined.

Color of anthocyanins was measured across a pH range of 1.5 to 8 for two genotypes. One milligram of pigment extracted and concentrated from Nain Noir and Neagra de Cluj was added to 100 milliliters of distilled water. The initial pH was 1.5 and was increased to 8 slowly with the addition of 4 percent NaOH solution. Color across the pH range was estimated by visual inspection.

RESULTS AND DISCUSSION

The black-hulled oil sunflower hybrid 894 yielded no anthocyanins. However, all purple-hulled genotypes yielded anthocyanins (Figure 1). The average anthocyanin concentration within the hulls of the six genotypes tested was 1216 milligrams per 100 grams hulls dry weight. The average pigment yield from sunflower hulls was 1.5 times the maximum reported yield of betanins from beets, 6 times the reported yield of anthocyanins from grape pomace, and 30 times the reported yield of anthocyanins extracted from cranberry pomace. While comparisons were made among sources of pigments on a dry weight basis, processors of grape and cranberry pomace and red beets must contend with raw material that is very high in moisture (60 to 90 percent.) Much time and expense is required to reduce moisture content of pigment extracts derived from the conventional sources. Stored sunflower hulls are 90 to 95 percent dry matter and the anthocyanins are already concentrated. Sunflower hulls should be far easier to process than wet grape and cranberry pomace or beet pulp.

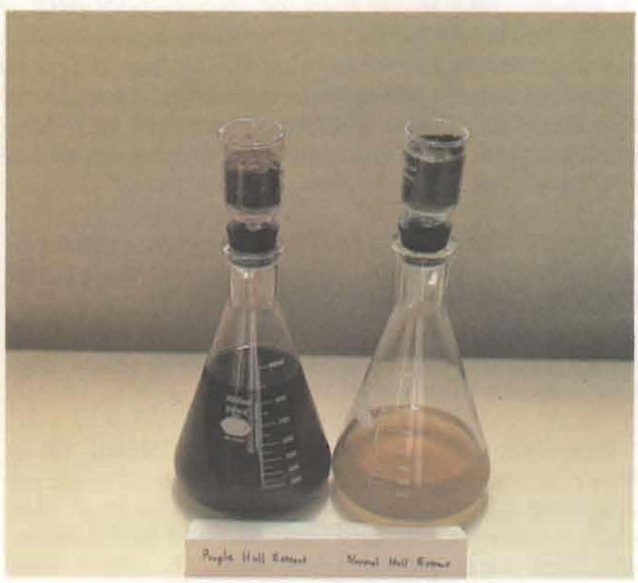

Figure 1. Extraction of anthocyanins with 95% ethanol-1.5N HCL (85:15) from purple sunflower hulls (left) and black sunflower hulls (right.)

The range of pigment concentration within the hull for the six genotypes tested was 0.64 to 1.59 percent (Table 1.) Sunflower achene samples used in this study were unreplicated in the field and obtained from two different locations. It was not possible to determine if variation between samples was due to genotypic or environmental influence. However, it is quite possible that certain environments and genotypes may modify the expression of this trait. Researchers have noted that while presence of anthocyanins in the hull is a qualitative characteristic, the intensity of color is a quantitative trait and differences between genotypes have been observed (11).

Table 1. Anthocyanins recovered from sunflower hulls of seven sunflower genotypes.

Genotype	Hull color	Anthocyanins recovered mg/100 g hulls	Anthocyanins recovered (%)
Hybrid 894	Black	0	0
Nain Noir	Purple	1585	1.59
Neagra de Cluj	Purple	1542	1.54
Nabhan	Purple	1340	1.34
Mars	Purple	1106	1.11
Hopi	Purple	1085	1.09
Navaho	Purple	638	0.64

Extraction yields were calculated on a dry weight basis.

Percentage of anthocyanins in three extracts was determined on a dry weight basis for Neagra de Cluj. Average anthocyanin concentration was 10.1 percent, range 9.8 to 10.3 percent. So extracts contained 90 percent non-anthocyanin materials. These materials were probably proteins, waxes, oils, and non-anthocyanin phenolic compounds. Pigment concentration within commercially marketed powders and concentrates is very low; for example, beet powders contain 0.3 to 1.0 percent pigment, 75 to 80 percent sugar, 8 to 10 percent ash, and 10 percent crude protein (7,10,14). The pigment concentration in the dried sunflower hull extract is 10 to 30 times the amount found in commonly marketed beet powders.

Absorbance profiles for the six purple-hulled genotypes were virtually identical (Figure 2). The TLC R_f values calculated for the six purple-hulled genotypes were quite similar (average was 81, range 80 to 83.) These results indicate that the anthocyanins of these six different genotypes grown at two locations are very similar or identical.

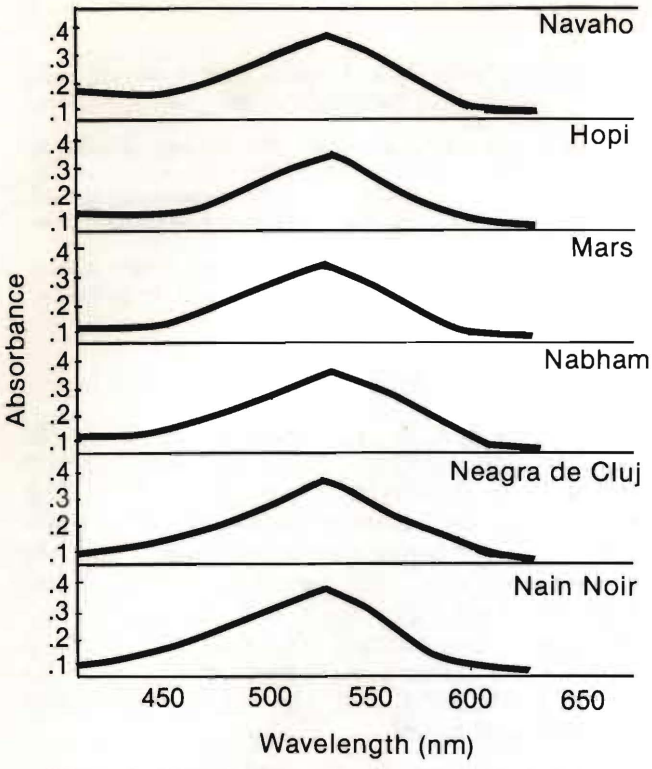

Figure 2. Absorbance spectrum of anthocyanin pigments extracted from six purple hulled sunflower genotypes.

The pH color stability of the pigments over variable pH was virtually identical for the two varieties tested (Neagra de Cluj and Nain Noir.) The important range for most food products is between pH 3 and pH 7. The anthocyanins derived from sunflower hulls are a shade of blue-red or purple color across this pH range (Figure 3.) The color of the anthocyanins was similar to grape anthocyanins (Table 2.) An ideal pigment like FD + C Red 2 would be completely stable across the pH range (3 to 7.) Anthocyanins, however, are very sensitive pH indicators. As pH is increased from 1.5 to 7, the anthocyanin pigments become increasingly bluish in hue (Table 2.) However, a chemical, rutin, may be used to stabilize anthocyanin color across pH ranges (16.) Betanin pigments are somewhat more stable across the pH range (3 to 7) than the anthocyanins.

The ease with which natural pigments can be extracted from source material is also an important consideration in choosing a source material. The anthocyanins of sunflower hulls can be extracted with water or ethanol with little or no preparation or processing of achenes. When sunflower achenes were soaked for three hours in acidified ethanol, 50 percent of the extractable anthocyanins diffused into the extractant. This represents a completely non-destructive extraction method in which neither hull or seed were visibly affected by pigment extraction. The other half of the hull pigments were recovered in the previously described manner in which hulls were separated from seed and pulverized. Anthocyanins can be extracted either from intact schenes prior to dehulling and crushing or from the hulls and protein meal remaining after oil extraction in a commercial oil crushing plant.

Table 2. Color of various natural pigments at eight different pHs.

	Observed color			
pH	Beet extract[1]	Grape skin extract[1]	Cranberry extract[1]	Sunflower hulls
1.5	——	——	——	Ruby red
2.0	——	——	——	Blue red
3.0	Ruby red	Purple red	Red	Blue red
4.0	Ruby red	Purple red	Brown red	Violet
5.0	Ruby red	Purple	Brick red	Violet
6.0	Ruby red	Purple	Brick red	Blue violet
7.0	Red violet	Violet	——	Blue
8.0	Violet	Blue	——	Green blue

[1]The color estimates for these extracts were taken from Riboh (1977).

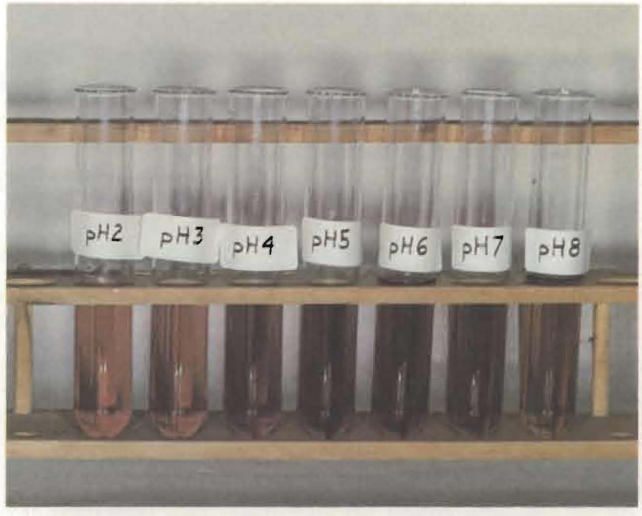

Figure 3. Color change of sunflower hull anthocyanin pigments in aqueous solution (1 mg pigment/100 ml H_2O) across the pH range of 2 to 8.

CONCLUSION

Anthocyanin pigments can be extracted easily from purple sunflower hulls in relatively large quantities and are very similar in color characteristics to those extracted from other natural sources (cranberry and grape.) These preliminary results indicate that purple sunflower hulls have practical potential as a source of anthocyanins to be used as food additives. Potential applications for sunflower anthocyanins include natural colors for jams, beverages, fruit yogurts, canned fruits, candy, confections, sherbets, pie fillings and toppings. However, FDA regulations, color stability and extraction methods need to be further evaluated. The ultimate economic significance of anthocyanins from sunflower hulls will depend on genetic transfer of the purple-hulled characters to oilseed sunflower hybrids grown in the large acreage in North Dakota, South Dakota, and Minnesota, whether such use could compete with other use alternatives, and whether any deleterious factor(s) might be associated with purple hulls.

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Guest Column continued

say we still have a lot to learn and a long way to go. Because rangeland has a much more complex species structure it is more difficult to understand. Each of these species contribute to the overall productivity of the range resource and this species composition changes from one range type to another.

This then is the task set before us at the Central Grasslands Research Station, to design research which will lead to a better understanding of the range ecosystem and to take this information and, using it, design management systems which will result in more beef production from native and introduced grasslands without damaging the resource.

The Botany Department in cooperation with Experiment Station staff are conducting a detailed vegetation survey of the native range sites on the station. A short duration grazing system which utilizes eight 40-acre pie-shaped pastures in a 4-5 day on, 35-40 day off rotation is being compared to season long grazing. A four-pasture rotation system on native range will evaluate a more conventional rotation schedule of 25-35 days on each of the four pastures. A third grazing system will evaluate the use of tame grases in a complementary grazing system where crested wheatgrass will be used for spring grazing, native range for early and mid-summer, Russian wildrye for late summer and early fall, and altai wildrye for late fall. These research trials will provide valuable data on vegetation and animal performance under different grazing systems.

In addition, small plot trials will evaluate new grass and legume varieties. Native species establishment and range fertilization trials will contribute to our knowledge of ways to improve tame grass production and to increase the forage production of native rangeland.

These research trials are by their design long term studies which take many years to complete. The producers of North Dakota are fortunate indeed to have a state government willing to make this long term commitment towards the understanding and improvement of this valuable natural resource.