Functional Properties of Pin-Milled and Air-Classified Dry Edible Bean Fractions[1]

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ABSTRACT

The functional properties of fractions separated by pin-milling and air classification of legumes were affected by heat treatment (roasting) of the legumes, the ratio of protein to starch in the fractions, and other constituents, such as the lipid content in the original legume samples. Nitrogen solubility index and foaming properties decreased as roasting temperature increased, most likely due to protein denaturation. Also, roasting resulted in increased water-holding capacity and cold paste viscosities. Cold paste viscosities of fractions from roasted legumes were notably higher than those from the nonroasted legumes. The starch-rich fractions showed the strongest water-holding capacity, nitrogen solubility, and cold paste viscosity, whereas the protein-rich fractions were superior in emulsification and foamability. Differences in functionality between the protein and starch fractions were not large in chick-pea, which exhibited poor starch and protein separation compared with pinto and navy beans. Although there were significant variations in some of the functional properties of each fraction with heat treatment, each food system may require fractions with different functional properties depending on the purpose. Therefore, it is not appropriate to say that a certain legume or air-classified fraction is more or less desirable.

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Functionality is any property of a food or a food additive, besides its nutritive ones, that affects its application and utilization in different food systems. However, with respect to legume utilization, only a few studies on milling of legumes and functionality of their flours in food products are reported (Hermansson 1979). Many functional properties are attributed to soybean fractions (Johnson 1970, Wolf 1970), but only a few studies on functional properties of the air-classified fractions of navy and pinto beans (*Phaseolus vulgaris* L.) and chick-pea (garbanzo bean) (*Cicer arietinum* L.) have been reported.

Functional properties of starch flour prepared from various legumes were studied by Schoch and Maywald (1968) and Naivikul and D'Appolonia (1979). Rheological properties of dough and baking quality of bread as affected by the addition of navy bean flour and protein concentrate were also studied (Sathe et al 1981a). Mittal and Usborne (1985) and Porteous and Wood (1983) reported that soybean flours and isolates are excellent emulsifiers and binders in high-fat foods, and these characteristics have been associated with their high water-holding capacity and fat absorption properties. Johnson (1970) reported that the nitrogen solubility levels of legume flours, which are frequently used as indicators of protein functionality and potential end use, were reduced as the extent of steaming increased.

The purpose of this study was to determine the functional properties of high-protein and high-starch fractions from nonroasted and roasted legumes fractionated by pin-milling and air classification for their potential suitability for use in food systems.

MATERIALS AND METHODS

Sample Preparation, Pin-Milling, and Air Classification

Three commercial dry legume samples, navy bean (Agri Sales Inc., Olivia, MN); pinto bean (ANV Export Corp., St. Johns,

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MI); and chick-pea (Harvest Gold Inc., Richardton, ND) were used in this research.

The sample preparation pin-milling and air classification procedures of dry edible bean seeds were carried out according to the method of Han and Khan (1990). After roasting in a particle-to-particle heat transfer type roaster (Aquilera et al 1982) and removing the hull from the samples, the double-pass procedure of pin-milling (Alpine Kolloplex laboratory pin mill, model 160 Z, Alpine American Corp., Natick, MA), followed by air classification (Alpine-Augsburg Mikroplex air classifier, type 132 MP, Alpine American Corp.) were used.

Physico-Chemical Properties

Physical and chemical properties were determined for the FI and FII (fine, protein-rich) and CII (coarse, starch-rich) fractions of the three legumes as described by Tyler et al (1981) and Han and Khan (1990). The yield, particle size index, color determination, moisture, protein, lipid, ash, amino acid composition, starch damage, and total dietary fiber content of all fractions were determined in duplicate and reported in an accompanying paper (Han and Khan 1990).

Water-Holding Capacity

The water-holding capacity was determined in duplicate by the method for protein materials as presented in AACC approved method 88-04 (1983). The sample weight was 5 g (dry weight basis) for all fractions. The measurements were made at room temperature (24°C) with double-distilled water. The results were reported as milliliters per gram of sample (ml/g) on a dry weight basis.

Nitrogen Solubility Index

The nitrogen solubility index (NSI) was determined according to AACC approved method 46-23 (1983) as modified by Bencini (1986). Double-distilled water (50 ml) was added to 1 g of legume sample and agitated for 2 hr in a 30°C water bath. The dispersed sample was centrifuged at 10,000 × *g* for 20 min. The protein content of 10 ml of the supernatant (which is 20%, w/v, of total protein content) was measured using a standard Kjeldahl method with antifoam agent according to method 46-11A (AACC 1983). The NSI was calculated as percent nitrogen present in the supernatant, and the factor 6.25 was used for the conversion to protein. The NSI was determined in duplicate at various pH values (pH 2.0–10.0) according to Betschart (1974).

Emulsion Capacity and Stability

The emulsion capacity was determined according to the method of Beuchat (1977) as modified by Sathe and Salunkhe (1981b). A 2-g sample was blended in a Waring Blendor, model 34BL97 (Dynamics Corp. of America, New Hartford, CT) with 100 ml of double-distilled water for 30 sec at high speed (approximately 3,500 rpm). Partially hydrogenated Mazola corn oil (Best Foods, CPC International Inc., Englewood Cliffs, NJ) was added in 5-ml portions with continued blending. The drop in consistency (from a maximum) judged by a decrease in resistance to blending (subjectively) was considered to be the point to discontinue adding oil. The amount of oil added up to this point was interpreted as the emulsifying capacity of the sample. The measurements were made at room temperature (24°C), and the results were reported as the average of two determinations.

The emulsion stability was determined by measuring the amount of water released from the optimum emulsion after centrifugation, according to the method of Johnson and Brekke (1983). The amount of sample required to emulsify 15 ml of oil was calculated from emulsion capacity; samples were diluted with double-distilled water at pH 7.0. Corn oil (15 ml) was added to the 15 ml of sample solution and emulsified using a Waring Blendor with a small adapter for 1 min at high speed (approximately 3,500 rpm). Each emulsion was placed in a graduated cylinder, and the total volume was recorded. The emulsions were stressed by centrifugation at 141 × *g* for 3 min at 24°C. Emulsion stability was expressed as the percent water released by the emulsion after centrifugation, and the results were reported as the average of two determinations.

The recorded results were calculated by the formula:

$$\% \text{ water released} = B/A \times 100$$

where, A = total volume of water in emulsion (milliliters), and B = volume of water released from emulsion after centrifugation (milliliters).

Foaming Capacity and Stability

Foaming capacity and foaming stability were determined in duplicate according to the modified method of Bencini (1986). Sample dispersions of 3% (w/v, dry weight basis) in double-distilled water were adjusted to pH 7.0 with 0.1*N* NaOH, and whipped for 5 min with a Sorvall Omni mixer, model 115 (Newton, CT) at high speed (10,000 rpm). The whipped sample was poured into a 250-ml graduated cylinder and the height of the foam recorded. The foam volumes were reported at time 0 (initial stage) and 10-, 30-, 60-, and 120-min intervals to study the foam stability of the samples. The results were expressed as a percent increase in volume and specific volume of sample (milliliters per gram).

The foaming capacity and stability of commercial soluble egg albumin powder (Fisher Scientific Co., Fairlawn, NJ) and commercial soybean protein isolate (Purina Protein 620, Ralston Purina Co., St. Louis, MO) were used as standards for comparison.

The results were calculated by the formula:

$$\text{Specific volume} = \text{vol 1 (ml)}/\text{wt 1 (g)}$$

$$\text{Volume increase (\%)} = (\text{vol 1} - \text{vol 2})/\text{vol 2} \times 100$$

where, wt 1 = weight after whipping (grams), vol 1 = volume after whipping (milliliters), and vol 2 = volume before whipping (milliliters).

Cold Paste Viscosity

The paste viscosity was determined in duplicate according to AACC approved method 22-10 (1983) as modified by Sosulski and Youngs (1979). A Brabender Viscoamylograph, type VA-1B, equipped with a 700-cm·g cartridge (C.W.C. Brabender Instruments Inc., South Hackensack, NJ) was used to study the pasting properties of the legume flour samples. A 50-g (dry weight basis) sample of legume fraction was suspended in 350 ml of distilled water by agitation in a Waring Blendor at low speed for 1 min. The slurry was poured at once into the viscoamylograph bowl, and the blender was rinsed with an additional 100 ml of water. The suspension was heated uniformly from 30 to 95°C (1.5°C/min increase), held at 95°C for 15 min, and then cooled uniformly to 50°C. The amylograms were interpreted and the pasting viscosity of fractions reported as Brabender units, based on temperature.

Statistical Analysis

Data from this study were analyzed with an IBM 3081D computer using the Statistical Analysis System (SAS) and Duncan's (1955) test as described by the SAS Institute (1985).

RESULTS AND DISCUSSION

Physical and Chemical Properties

The data for the physical and chemical property analyses are reported in another paper (Han and Khan 1990). Three fractions, FI and FII (high-protein) and CII (high-starch), were separated by pin-milling and air classification from navy beans, pinto beans, and chick-peas. The milling yield, particle size index, color difference, protein, lipid, ash, fiber, starch damage, and amino acid content were determined (Han and Khan 1990). These three fractions were used for evaluation of their functional properties reported in this study.

Functional Property Analyses

Water-holding capacity. It is known that water-holding capacity (WHC) by protein is a function of several parameters including size; shape; conformational characteristics; stearic factors; hydrophilic-hydrophobic balance of amino acids in the protein molecules, lipids, and carbohydrates associated with the proteins; thermodynamic properties of the system (energy of bonding, interfacial tension, etc.); physico-chemical environment (pH, ionic strength, vapor pressure, temperature, presence or absence of surfactant, etc.); and solubility of protein molecules (Chou and Morr 1979). However, polar amino groups of protein molecules are the primary sites of protein-water interactions that bind different amounts of water at cationic, anionic, and nonionic sites (Kuntz 1971).

In Table I, WHC values of the samples showed various trends. The chick-pea showed the lowest WHC in all fractions, due most likely to its lipid content, which is higher than in the other legumes (Han and Khan 1990). The roasted bean fractions showed higher values than the nonroasted bean fractions. This difference may be explained by differences in the finer particle size distribution of the roasted samples (Han and Khan 1990). Generally, the increase in WHC in roasted samples could be caused by the dissociation of proteins that might occur on heating and also by denaturation, even minimized by short-period treatment, which would unmask the nonpolar residues from the interior of the protein molecules (Abbey and Ibeh 1987).

NSI. The NSI was determined at several different pH values for each fraction as shown in Table II. The pH range was chosen with reference to the optimum range for food products, which is 2–10. In most cases, pH 4.0 resulted in the lowest nitrogen solubility, and the highest nitrogen solubility was at pH 10.0. The pH-solubility profile of fractions showed that minimum solubility occurred at pH 4.0, which is most likely the isoelectric (pI) region for all protein samples (Betschart 1974, Wang and Kinsella 1976). A minimum solubility range of pH 3–5 was reported by Lu and Kinsella (1972).

The CII fractions (high starch) showed slightly higher nitrogen solubilities than the FI and FII fractions (high protein) at most pH ranges, perhaps because of their lower protein contents. The pH dispersibility curves were determined from pH 2 to 10. In the fine fractions from all legume samples, 10–20% of the total nitrogen was soluble at pH 4.0, and solubility increased sharply beyond this region for most bean proteins. The results from this study showed trends very similar to those obtained by other researchers (Betschart 1974, Wang and Kinsella 1976). Particularly, the nitrogen solubility curve of the navy bean proteins was similar to the profiles reported by other authors for different legumes (Hang et al 1970, Betschart 1974).

The pinto bean and chick-pea samples gave high NSI values on both sides of the isoelectric pH of 4–5. Over the pH ranges studied, all fractions had very similar solubility properties. The nitrogen solubilities of leguminous seed proteins have been shown to be strongly pH dependent with a minimum point of nitrogen solubility at pH 4 (Plant and Tulsiani 1969, Hang et al 1970, Wolf 1970). Previous studies demonstrated that pH had a significant influence on functional properties (Sosulski and Youngs 1979). The roasted samples showed slightly lower NSIs than the nonroasted but the differences were not significant except for the coarse (CII) fractions (Table II).

It was not clear why the air-classified protein fractions showed distinctly lower nitrogen solubility values, in the range of 20–25% less than the original pin-milled fraction. The air-classified fractions exhibited poor dispersibility and this might have affected the nitrogen solubility values. Alternately, the grinding equipment might generate heat during the pin-milling operation, and the contact time of the flour with metal in the wide chamber pin mills might be sufficient to affect protein solubility.

Emulsion capacity and stability. The emulsion capacity of fractions was closely related to their lipid contents (lipid contents in Han and Khan 1990). Pin-milled chick-pea had the highest value followed by navy and pinto beans (Table III). This tendency was found in all fine and coarse fractions. In all cases these differences were significant. The treatment effect (roasting and nonroasting), however, did not significantly affect emulsion capacity of all sample fractions.

Emulsion capacity of the pin-milled flour of nonroasted pinto bean, navy bean, and chick-pea, respectively, was 17.50, 33.75, and 36.25 ml/g dry sample. The soy protein isolate (Purina Protein 620, Ralston Purina Co., St. Louis, MO), however, had lower

TABLE I
Duncan's Multiple Range Test for Water-Holding Capacity of Air-Classified Legume Flours and Their Fractions

Variables	*n*	Mean[a] (ml/g)
Legume effect		
Navy	8	1.28 b
Pinto	8	1.53 a
Chick-pea	8	1.00 c
Treatment effect		
Nonroasted	12	1.19 a
Roasted	12	1.35 a
Fraction effect[b]		
Pin-milled	6	1.12 b
FI	6	1.31 ab
FII	6	1.40 a
CII	6	1.26 ab

[a]Means with the same letter are not significantly different ($P = 0.05$).
[b]FI = First fine fraction; CII and FII = coarse and fine fractions, respectively, from remilling of first coarse fraction.

TABLE II
Duncan's Multiple Range Test for Nitrogen Solubility Index of Air-Classified Legume Flours and Their Fractions

		Mean[a] (%)			
Variables	*n*	Pin-Milled	FI	FII	CII
Legume effect					
Navy	8	61.51 a	53.07 a	56.36 a	69.02 a
Pinto	10	62.49 a	56.33 a	58.01 a	61.54 a
Chick-pea	12	55.91 a	52.24 a	66.86 a	61.35 a
Treatment effect					
Nonroasted	15	59.06 a	55.18 a	63.42 a	67.36 a
Roasted	15	60.13 a	52.58 a	58.80 a	59.55 b
pH effect					
2.0	6	74.05 b	59.96 b	72.18 b	79.41 b
4.0	6	17.77 c	12.31 d	15.70 c	19.62 c
6.0	4	28.89 c	27.13 c	27.00 c	28.96 c
7.0	6	68.91 b	70.78 b	76.73 ab	80.65 b
8.0	2	74.31 b	84.71 a	87.78 ab	77.05 b
10.0	6	93.22 a	92.80 a	93.67 a	92.62 a

[a]Means in the same column followed by the same letter are not significantly different ($P = 0.05$). FI = First fine fraction; CII and FII = coarse and fine fractions, respectively, from remilling of first coarse fraction.

TABLE III
Duncan's Multiple Range Test for Emulsion Capacity and Stability of Air-Classified Legume Flours and Their Fractions

Variables	*n*	Capacity Mean[a] (ml/g)	Stability[b] Mean[a] (ml/g)
Legume effect			
Navy	8	33.75 b	37.59 b
Pinto	8	26.25 c	64.38 a
Chick-pea	8	42.81 a	25.34 c
Treatment effect			
Nonroasted	12	34.79 a	45.64 a
Roasted	12	33.75 a	39.23 a
Fraction effect[c]			
Pin-milled	6	28.96 c	42.12 a
FI	6	46.25 a	39.10 a
FII	6	38.75 b	40.22 a
CII	6	23.13 d	48.30 a

[a]Means in the same column followed by the same letter are not significantly different ($P = 0.05$).
[b]Values are expressed as the percentage of water retained in the emulsion after centrifugation.
[c]FI = First fine fraction; CII and FII = coarse and fine fractions, respectively, from remilling of first coarse fraction.

capacity (43.25 ml/g) than FI values shown in Table III. The reason for this unexpected low emulsion capacity value was perhaps the high protein and low oil contents of soybean protein isolate. Differences between samples in oil emulsification capacities were not large, which may reflect the small sample size used in this study. The emulsification value for soybean protein was similar to those of the protein fractions examined in this study. The formation and stability of an emulsified oil droplet depends on the formation of a charged layer around the droplet causing repulsion and/or the formation of a film around the droplet by solutes such as proteins (Kinsella 1976). Hydrophobic regions of protein molecules associate at the lipid interface while polar and ionic regions associate with the aqueous phase (Johnson and Brekke 1983).

Emulsion stability was measured by the percentage of water released after centrifugation. There were significant differences among the three legumes (Table III). In contrast to emulsion capacity, pinto bean (lowest emulsion capacity) shows the highest percent water released, which is poor emulsion stability, followed by navy bean and chick-pea. Roasting did not significantly affect the capacity and stability. The stability of the fine and coarse fractions did not show a significant difference, in contrast to emulsion capacity.

By possessing the capacity to lower the interfacial tension between hydrophobic and hydrophilic components in foods, many proteins are effective surface-active agents. Functions performed in this role include emulsion and foam formation. Various factors and conditions influence the measurement of emulsifying capacities of proteins, among these are equipment design, rate of oil addition, temperature, pH, protein source, solubility and concentration, kind of oil used, salt, sugar, and water content. Therefore, emulsifying capacity is not solely a property of the protein under test but is rather a property of the emulsion system, equipment, and method used to produce the emulsion (Pearce and Kinsella 1978).

Foaming capacity and stability. Foaming capacity of each fraction was expressed by two different methods, percent volume increase and specific volume (milliliters per gram) of foam after foaming, as shown in Table IV. Roasting beans significantly lowered their foaming capacity. Also, chick-pea, with its high lipid content, showed the lowest foaming capacity, as expected. The difference between the fine and coarse fractions was not significant. The fine fractions exhibited excellent foaming capacity and stability, except for chick-pea, compared with the soybean control. These properties suggest potential applications in meat emulsions, beverages, and bakery products (Sosulski and McCurdy 1987).

The foaming stability was determined by the volume decrease (milliliters) of foam with elapsed time after foaming (Table V). Generally, the foaming stability showed a trend similar to foaming capacity. Volume changes for the high-protein fractions were less dramatic than for the high-starch fractions. Navy and pinto bean fractions developed high initial foam volumes and maintained their relatively coarse foam structure throughout the protein periods of holding. Chick-pea lost its foam faster than the navy and pinto beans. Also, roasting significantly affected the foaming stability of all fractions. All these effects were significant, as shown in Table V. An important functional requirement of proteins used in angel food cake, whipped toppings, divinity and souffle-like products is the capacity to form stiff, stable foams (Waniska and Kinsella 1979).

Cold paste viscosity. The amylograms are the plots for the corrected viscosity. The data are summarized in Table VI. With the exception of the fine fractions, all other samples showed similar patterns. The change in viscosity after holding for 15 min at 95°C was rapid, except for the fine fractions. As can be seen from Table VI, no values are reported for peak viscosity because, unlike wheat flour, no distinct peaks were obtained with the legume flours.

This cold paste viscosity method was originally used to evaluate the starch granule itself. In this study, fine fractions clearly showed low viscosity (Brabender unit) values similar to low values of starch damaged pattern. Both roasted and nonroasted chick-pea, however, showed large differences between the first and second fine fractions with the nonroasted samples showing the larger difference. The values of roasted fractions of navy and pinto beans also showed higher cold paste viscosity than nonroasted fractions.

High peak values at the end of the cooling cycle in the visco-amylograph curves were primarily a property of the starch fractions, and very low values were obtained for chick-pea flours as well as most protein concentrates. Intermediate hot viscosity values combined with high cold-paste viscosity were characteristic of navy bean and pinto bean starch fractions (Sosulski and Youngs 1979). This viscosity behavior of the fractions was characteristic of their starch type. Therefore, further research will be necessary for an explanation of viscosity behavior of the bean starch granules (Sathe and Salunkhe 1981b). A direct comparison between the present amylogram data of the various legume flours and previously reported data cannot be made.

CONCLUSIONS

Data presented showed that a portion of the variation in functional properties among legume flours and air-classified fractions can be ascribed to the ratio of protein to starch, and other constituents, such as lipids, in the original flour.

Functional properties as well as physical and chemical properties are expected to be dependent on the degree of heat

TABLE IV
Duncan's Multiple Range Test for Foaming Capacity of Air-Classified Legume Flours and Their Fractions

Variables	*n*	Volume Increase Mean[a] (%)	Specific Volume Mean[a] (ml/g)
Legume effect			
Navy	8	111.25 a	2.15 a
Pinto	8	106.38 a	2.11 a
Chick-pea	8	53.75 b	1.57 b
Treatment effect			
Nonroasted	12	108.17 a	2.12 a
Roasted	12	72.75 b	1.76 b
Fraction effect[b]			
Pin-milled	6	83.33 a	1.86 a
FI	6	100.00 a	2.04 a
FII	6	96.17 a	2.00 a
CII	6	82.33 a	1.86 a

[a] Means in the same column followed by the same letter are not significantly different ($P = 0.05$).
[b] FI = First fine fraction; CII and FII = coarse and fine fractions, respectively, from remilling of first coarse fraction.

TABLE V
Duncan's Multiple Range Test for Foaming Stability of Air-Classified Legume Flours and Their Fractions

Variables	*n*	Mean[a] (ml)
Legume effect		
Navy	40	121.23 b
Pinto	40	130.55 a
Chick-pea	40	43.78 c
Treatment effect		
Nonroasted	60	118.07 a
Roasted	60	78.97 b
Fraction effect[b]		
Pin-milled	30	91.67 b
FI	30	104.97 a
FII	30	105.13 a
CII	30	92.30 b
Time effect (min)		
0	24	138.04 a
10	24	104.96 b
30	24	92.42 c
60	24	84.04 c
120	24	73.13 d

[a] Means with the same letter are not significantly different ($P = 0.05$).
[b] FI = First fine fraction; CII and FII = coarse and fine fractions, respectively, from remilling of first coarse fraction.

TABLE VI
Cold Paste Viscosity of Pin-Milled Legume Flours and Air-Classified Fractions[a]

Fractions[b]	Temperature at 10 BU (°C)	Peak Height (BU)	15-min Height[c] (BU)	50°C Height[d] (BU)	95°C Height (BU)
Navy					
Pin-milled	72	740	460	740	270
FI	75	245	50	245	50
FII	69	225	110	225	75
CII	74	900	640	900	485
R-Navy					
Pin-milled	75	820	470	820	285
FI	75	230	45	230	60
FII	77	230	45	230	55
CII	75	1,110	685	1,110	515
Pinto					
Pin-milled	72	570	445	570	310
FI	52	245	100	245	85
FII	60	285	265	255	195
CII	74	855	650	855	470
R-Pinto					
Pin-milled	75	675	290	675	200
FI	66	280	50	280	50
FII	72	300	110	300	95
CII	75	900	540	900	425
Chick-pea					
Pin-milled	72	510	445	510	485
FI	90	100	50	100	40
FII	75	305	240	305	195
CII	71	635	500	635	535
R-Chick-pea					
Pin-milled	74	495	390	495	410
FI	84	80	50	80	50
FII	81	160	130	160	130
CII	72	680	480	680	485

[a]Values reported are an average of two determinations and are on a dry weight basis.
[b]FI = First fine fraction; CII and FII = coarse and fine fractions, respectively, from remilling of first coarse fractions. R = roasted dry edible beans.
[c]Viscosity of the correlated starch curve in Brabender units at the end of a 15-min period of holding at 95°C.
[d]Viscosity at 50°C in Brabender units during cooling cycle.

treatment (roasting). In most cases, NSI and foamability decreased as product temperature increased. Protein insolubilization and denaturation are believed to be responsible for this effect. Roasted products showed increased WHCs and cold viscosities. Cold paste viscosities of roasted products were notably higher than those of the nonroasted product. Therefore, dry-roasting treatment seems worthy of more detailed study as an inexpensive way to process beans at the harvest point to partially inactivate antinutritional factors (Aguilera et al 1982) and to preserve desirable quality characteristics of beans for longer periods. Moreover, the coarse fractions (starch rich) showed the strongest WHC, NSI, and cold paste viscosity, whereas the fine fractions (protein rich) were superior in emulsification and foamability. Differences in functionality between the protein and starch fractions were not large in chick-pea, which exhibited poor starch and protein fractionation.

Consequently, food and industrial processors require ingredients with weak, intermediate, or strong functional properties, depending on end use. Therefore, it is not appropriate to designate a particular air-classified fraction as being superior to another, i.e., the end use would dictate the desirability of a particular fraction.

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