antiserum is an asset that provides the experimenter with great flexibility.

The increased sensitivity, selectivity, and tracer stability of the assay reported here permits studies to be done on the biosynthesis and regulation of naringin and other flavanone 7-neohesperidosides, particularly in controlled systems such as tissue culture. The assay is well suited for the analysis of numerous samples such as in monitoring programs during juice processing and for screening of whole fruits, seedlings, individual callus cultures, and even entire groves. Thus, it is now possible to analyze the effects of nutrients, geographic distribution, root stocks, cultivation methods, and freezes on the production and accumulation of naringin and related flavanone 7-neohesperidosides in citrus fruits at the population level.

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Contents of Detergent-Extracted Dietary Fibers and Composition of Hulls, Shells, and Teguments of Almonds (*Prunus amygdalus*)

Fulgencio Saura-Calixto,* Jaime Cañellas, and Juan García-Raso

The contents of acid and neutral detergent fibers (ADF and NDF) and their fractions—hemicellulose, cellulose, and lignin—in hulls, shells, and teguments of almond fruit were determined. Pectins, protein, tannins, and acid polysaccharides were found in ADF and NDF residues. Some nutritional aspects of these subproducts in animal nutrition are considered. Determinations of polyphenols, pectins, gums and mucilages, mineral elements, oil, soluble sugars, and nitrogen were also carried out on hulls, shells, and teguments.

The fruit of the almond is made up of four parts. The mesocarp (hull), which opens when the fruit ripens, is the most external. The endcarp (shell) is hard and woody and contains one or two kernels covered by a thin brown skin called the tegument. Using average values determined by the authors for 14 almond varieties cultivated on the island of Mallorca, Spain, these parts represent the following percentages of weight for the whole fruit: hulls, 32.5; shells, 47.9; teguments, 1.2; kernels, 18.4.

According to 1981 data (El Campo, 1982) Spanish production was about 75 000 tons of almond kernels, which represent approximately 124 000 tons of hulls, 183 000 tons of shells, and 4600 tons of teguments. These subproducts have very few practical applications today.

Aside from obtaining furfural from the shells, which is a well-known process, Lopez-Gonzalez et al. (1976), Berenguer et al. (1977), and Linares-Solano et al. (1980) have obtained active carbons from this subproduct and amply studied their properties. Shells have also been used for preparing several other products. Among them are xylose and xylitol (Nobile, 1971), a stop-leak for automobile cooling systems (Lasswell and Monier, 1967), a fluid with bactericidal properties (Martín et al., 1965), fuel gas and charcoal (Sachdev, 1974), and a substance to increase microbial growth in soil (Choroszy et al., 1981).

Velasco et al. (1965), Ohanesian et al. (1973), Sanchez-Vizcaino and Moreno-Rios (1978), and Alibés et al. (1979) have studied the application of almond subproducts for animal feeding. Farmers of Mallorca have traditionally employed hulls for animal nutrition and used shells as a fuel source.

Copious information concerning the composition of almond kernels exists. In previous papers, Saura-Calixto et al. (Saura-Calixto et al., 1980, 1981, 1982; Saura-Calixto and Cañellas, 1982a) studied the soluble sugars, general composition, mineral elements, protein, and amino acids of the kernels, referring to data of other authors and comparing results. Few references in the literature con-

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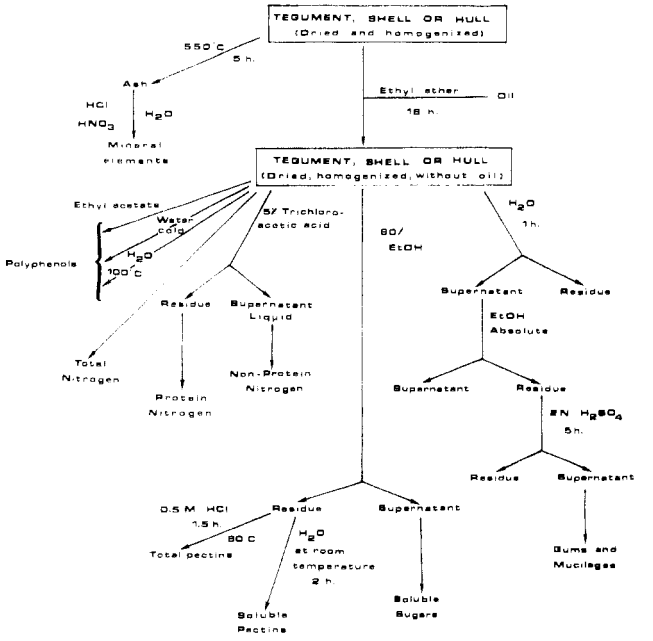

Figure 1. Simplified diagram of experimental procedures carried out on hulls, shells, and teguments.

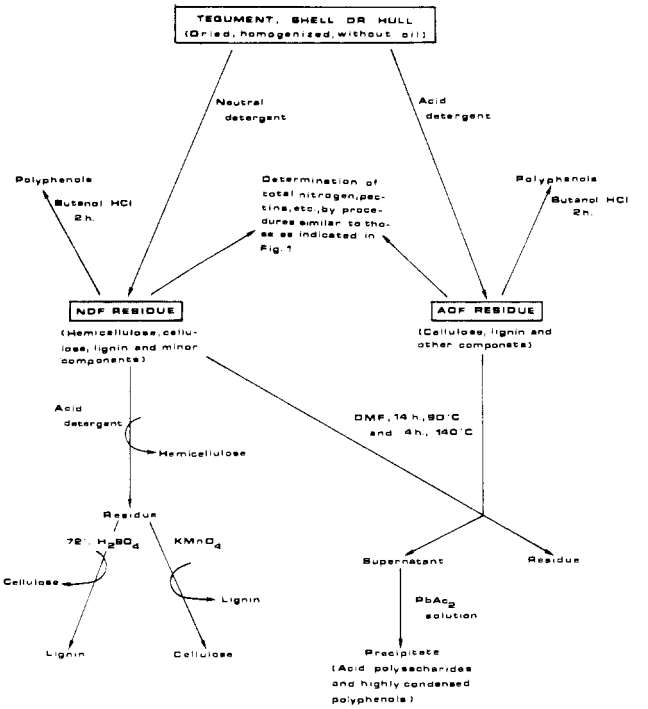

Figure 2. Simplified diagram of experimental procedures carried out on NDF and ADF residues of hulls, shells, and teguments.

cerning the subproducts of the fruit, however, are to be found. Several authors have studied components of almond hulls: soluble sugars (Sequeira and Leiw, 1970), gums and mucilages (Ryugo and Labavitch, 1978), volatile components (Buttery et al., 1980a,b), and general composition (Saura-Calixto and Cañellas, 1982b). We do not, however, find specific references on the chemical composition of shells and teguments.

EXPERIMENTAL SECTION

Samples used corresponded to the comercial product named "Mallorca propietor" made up of a mixture of the principal almond varieties cultivated on the island. Samples were homogenized and ground to particles of ≤0.5 mm and moisture was removed by drying at a temperature of 100 °C. The oil was extracted with ethyl ether by using a Soxhlet extractor. Determinations of mineral elements were carried out on HCl/HNO_3 solutions of ashed samples.

Hulls, shells, and teguments were treated with 5% trichloroacetic acid, and non-protein and protein nitrogen were determined in the supernatant and residue, respectively. Soluble sugars were determined on extracts obtained with 80% ethanol. Pectins were determined on solutions obtained by treating samples without soluble sugars with water at room temperature and 0.5 M HCl at 80 °C. Ethanol was added to the aqueous extract to produce a precipitate. The residue was treated with 2 N H_2SO_4 for 5 h, and determinations of gums and mucilages were carried out on the hydrolyzed product. Extractions with ethanol, ethyl acetate, water, and BuOH/HCl were employed to determine polyphenols. Extraction with dimethyl formamide in a nitrogen atmosphere was made to determine acid polysaccharides and highly condensed polyphenols.

Samples were also treated with neutral and acid detergent solutions, respectively, to determine NDF and ADF contents. Hemicellulose content was determined as the weight loss of the NDF residue when treated with acid detergent. The residue was treated with $KMnO_4$ and 72% H_2SO_4 to determine cellulose and lignin contents, respectively.

Figures 1 and 2 show the treatments carried out. Analytical procedures used were the following: moisture, ash, oil, and nitrogen (semimicro-Kjeldhal method), AOAC methods (1980); soluble sugars, Haas colorimetric method with anthrone, modified by Yadey and Weisler (Snell and Ettre, 1971); Mg, Ca, Fe, Cu, Mn, and Zn by atomic absorption and K by emission according to the specifications of Pinta (1971); phosphorus by colorimetry in the form of vanadium phosphomolybdate (Pearson, 1976); pectins, with the carbazole method (McComb and McReady, 1952) using galacturonic acid as a standard [the procedures described by Würsch (1980) and Primo Yufera (1979) were also used]; polyphenols, by titration with $KMnO_4$ in accordance with AOAC (1980) methods, using D-catechin as the standard; gums and mucilages, by the procedure described by Ryugo and Labavitch (1978), analyzed as glucose units; ADF, NDF, hemicellulose, cellulose, and lignin, by the procedure of Van Soest (1973, 1976).

A Perkin-Elmer 703 atomic absorption spectrophotometer was used for analysis of the mineral elements, with the exception of phosphorus. Colorimetric analysis were carried out with a Spectronic 21 Bausch & Lomb colorimeter. The remaining test were carried out with a conventional laboratory apparatus.

RESULTS AND DISCUSSION

All results are average values of assays made on five different samples. Chemical composition of hulls was studied in a previous paper (Saura-Calixto and Cañellas, 1982b) showing a considerable amount of soluble sugars, 26.55%, while oil, protein, and ash contents are 3.34%, 2.7%, and 6.09%, respectively, expressed on dry matter basis.

Results of determinations corresponding to test indicated in Figure 1 are shown in Tables I and II. As can be observed, the subproducts have appreciable amounts of non-protein nitrogen. Only the tegument has considerable protein and oil contents.

The high content of ash in hulls and teguments should be mentioned. Amounts of mineral elements in teguments and shells, determined by absorption and colorimetry, are included in Table I. The high concentration of calcium in teguments, together with the low content of phosphorus

Table I. Chemical Composition of Hull, Shell, and Tegument (Percent with Respect to Dry Matter, Except for Mineral Elements, mg/100 g of Dry Matter)[a]

	hull[b]	shell	tegument
moisture	11.90 ± 0.35	12.40 ± 0.30	12.10 ± 0.31
fat	3.34 ± 0.19	0.41 ± 0.05	14.30 ± 0.32
soluble sugars	26.55 ± 0.46	0.35 ± 0.04	3.66 ± 0.12
ash	6.09 ± 0.12	0.69 ± 0.03	4.05 ± 0.10
mineral elements			
potassium	2147 ± 25	163 ± 5	737 ± 12
calcium		129 ± 5	613 ± 14
magnesium	157 ± 3	16 ± 0.7	188 ± 3
phosphorus	128 ± 2	16 ± 0.5	265 ± 3
iron	14.00 ± 0.20	4.52 ± 0.14	11.27 ± 0.25
zinc	1.24 ± 0.20	5.91 ± 0.11	6.12 ± 0.10
manganese	0.84 ± 0.01	0.30 ± 0.01	3.76 ± 0.08
copper	0.42 ± 0.02	0.91 ± 0.03	1.23 ± 0.03
nitrogen			
total nitrogen	0.658 ± 0.004	0.35 ± 0.01	1.90 ± 0.04
non-protein nitrogen	0.22 ± 0.02	0.20 ± 0.02	0.27 ± 0.03
protein nitrogen	0.43 ± 0.02	0.16 ± 0.02	1.68 ± 0.05
protein (protein N × 6.25)	2.70 ± 0.12	1.00 ± 0.12	10.50 ± 0.31

[a] Average value ± $ts/n^{1/2}$ (t = Student's t value; $\alpha = 0.05$; s = standard deviation; $n = 5$, number of assays). [b] Obtained from a previous paper (Saura-Calixto and Cañellas, 1982b).

Table II. Polyphenols, Pectins, Gums, and Mucilages of Almond Subproducts (Percent with Respect to Dry Matter)[a]

	hull	shell	tegument
polyphenols (as D-catechin)			
extract AcOEt	0.02 ± 0.00	0.01 ± 0.00	0.13 ± 0.01
extract EtOH	0.35 ± 0.03	0.03 ± 0.01	1.96 ± 0.12
extract H_2O (20 °C)	2.85 ± 0.08	0.10 ± 0.01	1.25 ± 0.09
extract H_2O (100 °C)	6.02 ± 0.20	0.48 ± 0.05	3.03 ± 0.21
pectins (as galacturonic acid)			
extract H_2O (20 °C)	1.28 ± 0.10	0.24 ± 0.01	0.35 ± 0.02
totals	3.98 ± 0.22	0.58 ± 0.04	3.33 ± 0.15
gums and mucilages (as glucose)	0.086 ± 0.004	0.009 ± 0.002	0.225 ± 0.017

[a] Average value ± $ts/n^{1/2}$.

Table III. Fiber Content and Fractionation in Almond Subproducts (Percent with Respect to Dry Matter)[a]

	hull	shell	tegument
NDF	28.02 ± 1.04	90.31 ± 1.68	46.43 ± 1.56
ADF	28.83 ± 1.15	61.96 ± 1.45	45.24 ± 1.90
hemicellulose	10.03 ± 0.49	29.13 ± 0.69	12.19 ± 0.60
cellulose	11.93 ± 0.64	34.08 ± 0.99	21.90 ± 1.12
lignin	7.05 ± 0.51	27.80 ± 1.12	10.01 ± 0.63

[a] Average value ± $ts/n^{1/2}$.

and magnesium in shells, usually macronutrients in vegetable matter, should be pointed out. Specific references concerning mineral composition of teguments and shells have not been found by us.

The high content of potassium, 2.147 mg/100 g of dry matter, in hulls should be mentioned (Saura-Calixto and Cañellas, 1982b). Engwall (1977) also cites a high concentration of potassium and a low content of calcium in this subproduct, although numerical data are not reported.

Hulls have the highest content of polyphenols and shells the lowest. Except for those in the teguments, the principal polyphenols are only very slightly soluble in common organic solvents, indicating that the tannins are highly condensed.

Pectin content is high in the teguments and hulls and very low in shells. Hulls have the highest percentage of water-soluble pectins. Gum and mucilage concentration is very low in all cases. Ryugo and Labavitch (1978) found an amount of 44 mg/100 g of these components in hulls of the Mediterranean variety named Marcona.

Dietary Fiber. A principal application of these subproducts is animal nutrition. Since fiber components are considered to have an important role in nutrition (Twelfth International Congress of Nutrition, 1981; Symposium on Dietary Fiber (1980), 1981], we considered it important to determine the composition of this fraction. Results are included in Table III. As can be observed, the difference between NDF and ADF coincides with the hemicellulose content in shells. This is not the case for the hulls and teguments, which may be due to the presence, particularly in ADF, of other components cited in the literature such us protein, polyphenols, pectins, gums and mucilages, and acid polisaccharides (Van Soest and Robertson, 1979; Belo and de Lumen, 1981; Ryugo and Labavitch, 1978; Würsch, 1979). To veryify this, many analysis were carried out on ADF and NDF fractions, which are indicated in Figure 2. Results are listed in Table IV. Determination of gums and mucilages was omitted because the concentration in the subproducts were quite low.

Table IV. Other Undigestible Compounds in Dietary Fiber (Percent with Respect to Dry Matter)[a]

	hull		shell		tegument	
	NDF	ADF	NDF	ADF	NDF	ADF
total nitrogen	0.07 ± 0.01	0.13 ± 0.01	0.08 ± 0.01	0.12 ± 0.01	0.21 ± 0.02	0.68 ± 0.05
total pectins	0.13 ± 0.02	0.31 ± 0.04	0.08 ± 0.01	0.26 ± 0.03	0.31 ± 0.03	0.88 ± 0.09
polyphenols (extract BuOH/HCl)	0.34 ± 0.05	2.87 ± 0.18	negligible	negligible	0.36 ± 0.04	1.87 ± 0.11

[a] Average value ± $ts/n^{1/2}$.

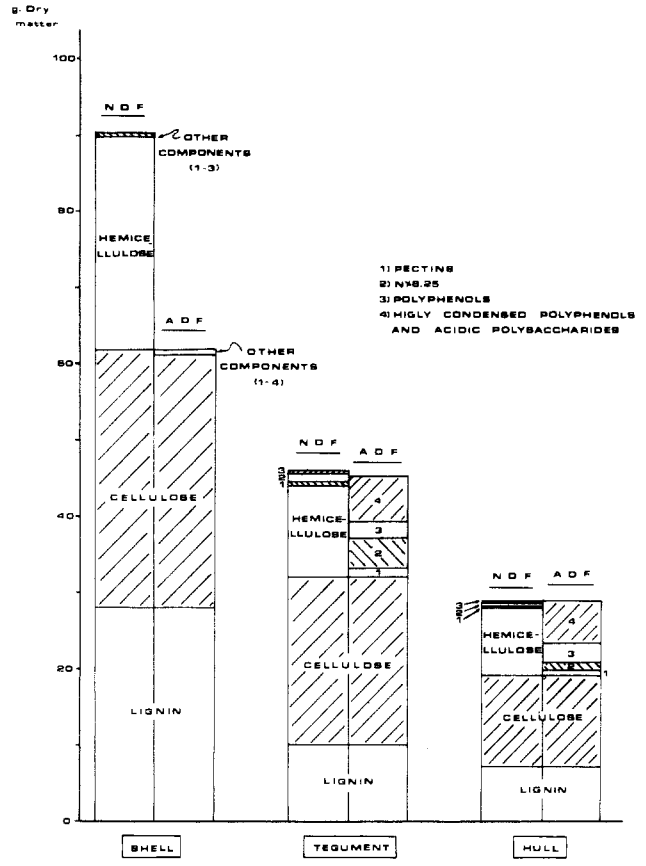

Figure 3. Distribution of components in NDF and ADF residues of almond subproducts.

Low concentration of these components in ADF and NDF fractions of shells are in agreement with the fact that hemicellulose concentration can be determined as the difference between NDF and ADF.

Appreciable amounts of protein, pectins, and polyphenols remain in the ADF of hulls and teguments, since these components are lower in NDF. Since a sum of the amounts of cellulose, lignin, protein, pectins, and polyphenols found in ADF was lower than the weight of ADF, we concluded that the fraction remained undetermined. For this reason an extraction with dimethylformamide in a nitrogen atmosphere was made under the conditions indicated in Figure 2, resulting in a high content of extracted substances. These components precipitate in lead acetate, and in agreement with Würsch (1979), we conclude that they are highly condensed tannins and acid polysaccharides. A similar assay was made on NDF showing negligible amounts of extracted substances. Results are also shown in Figure 3.

Hulls, shells, and teguments have a gross energy value of the same order as other products used in animal nutrition. Several authors (Velasco et al., 1965; Sanchez-Vizcaino and Moreno-Rios, 1978; Alibés et al., 1979; Ohanesian et al., 1973) have studied the nutritional value of these subproducts. Table V lists characteristic parameters of hulls, shells, and teguments for ruminants. The table includes data obtained by the cited authors as well as data empirically calculated by us using the methods described by Crampton and Harris (1979) and McDonald et al. (1979).

Low values of total digestible nutrients (TDN) for shells indicate their poor nutritional value. It follows that values for digestibility, digestible energy, and metabolizable energy are also low. Energy values for hulls and teguments are moderate, but digestibility values of both can be considered low, especially for the protein fraction. This is related to the formation of tannin–protein complexes, especially in hulls, which has a negative effect on digestibility (Mitjavila, 1980). For this reason the utilization of these subproducts mixed with other ingredients is proposed. Alibés et al. (1979) and Sanchez-Vizcaino and Moreno-Rios (1978) have studied this question and propose mixing with molasses, urea, and alfalfa.

Table V. Nutritional Values of Hull, Shell, and Tegument[a]

	hull	shell	tegument
gross energy (GE), kcal/kg	4560	4288[b]	4960[b]
total digestible nutrients (TDN), g/100 g	57.7	20.7	49.5
coeff of apparently digestible protein, %	essentially 0	essentially 0	10.4
digestible energy (DE), kcal/g	2540	989	2280[b]
metabolizable energy (ME), kcal/kg	2270	791	1780

[a] From Engwall (1977), Velasco et al. (1965), Sanchez-Vizcaino and Moreno-Rios (1978), and Alibes et al. (1979). [b] Data empirically calculated.

Registry No. Hemicellulose, 9034-32-6; cellulose, 9004-34-6; lignin, 9005-53-2; pectin, 9000-69-5; nitrogen, 7727-37-9.

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Mineral Composition of Vegetable Crops Fertilized with Fish-Soluble Nutrients

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Pea, tomato, radish, and lettuce crops were analyzed for 20 elements by atomic absorption spectrometry. Elemental contents of vegetables that had been fertilized with different rates of fish-soluble nutrients were compared to those fertilized with standard Hoagland nutrient solution. The mineral contents of the edible organs were altered by fertilization with fish-soluble nutrients. Pea seeds and tomato fruits responded favorably, with no heavy metal accumulation. Lettuce leaves and radish storage roots showed a greater accumulation of certain mineral elements but no excessive heavy metal accumulation.

Fish and its byproducts have been recognized and used as fertilizer (Aung and Flick, 1980; Ceci, 1975; Emino, 1981; Van Breedveld, 1969). The fish-soluble nutrients are complex mixtures of minerals, amino acids, fats, and vitamins derived from the menhaden industry after oil removal (Soares et al., 1973). The general crop responses of plants fertilized with fish-soluble nutrients have been favorable (Aung et al., 1981). However, the effects of fish-soluble nutrients on possible chemical compositional changes of food crops are not well documented, and the lack of information limits the commercial use of fish solubles as a fertilizer. Since vegetable crops are predominantly consumed to provide essential minerals in the diets (Keane, 1972; Senti and Rizek, 1975), several of the important vegetables were selected for determining the effects of fish-soluble nutrients on the mineral composition of the edible organs. This paper reports the mineral contents of pea, tomato, radish, and lettuce crops fertilized with fish-soluble nutrients.

MATERIALS AND METHODS

Crop Growing. Seeds of pea (*Pisum sativum* L. cv. Little Marvel), tomato (*Lycopersicon esculentum* Mill. cv. Fireball), lettuce (*Lactuca sativa* L. Buttercrunch), and radish (*Raphanus sativus* L. cv. Cherry Belle) were sown in a sand medium contained in pots under greenhouse conditions. Pea, lettuce, and radish were grown during the spring with 18 °C night and 24 °C day temperatures. Tomato was grown in late spring and early summer months at temperatures of 21 °C night and 28 °C day. Pea was grown in 9 cm diameter plastic pots, radish was grown in 13 cm diameter clay pots, and tomato and lettuce were grown in 18 cm diameter clay pots. The sand medium (All Star Concrete Co., Blacksburg, VA 24060) used had the following properties: pH 8.1; NO_3–N, 5 ppm; P_2O_5, 4 ppm; K_2O, 11.5 ppm; CaO, 571 ppm; MgO, 30 ppm; 0.1% organic matter; 230 ppm of soluble salts (1:2 soil to water extract). The plants were fertilized at designated intervals (see the tables) with (a) various concentrations of fish solubles (Zapata Haynie Corp., Reedville, VA 22539) and (b) complete nutrient solution 1 prepared according to Hoagland and Arnon (1950) as a reference standard for gauging the relative effectiveness of the fish solubles. A randomized complete design of 8–10 replicates was used. The crops were grown to maturity and the edible parts harvested for determination of mineral nutrients composition.

Sample Preparation. Peas were removed from the pods, sliced in half, and oven-dried for 18 h at 95 °C. Tomato samples were sliced in half, and the seeds were separated from the pulp and then dried for 18 h at 100 °C in a forced draft oven. Radish roots and tops and lettuce leaves were lyophilized for 48 h. The dry samples of the selected crops were ground in a miniblender for 3 min and analyzed for mineral nutrients composition.

Mineral Nutrients Determination. A wet ashing procedure was used for atomic absorption spectrometry (AAS) analyses (Simpson and Blay, 1966). A Perkin-Elmer Model 403 atomic absorption spectrophotometer was used. A 4-in. burner head and standard air–acetylene flame were used for determination of calcium, copper, chromium, iron, magnesium, manganese, nickel, potassium, sodium, zinc, and the heavy metals cadmium, cobalt, lead, mercury, and silver. For barium, boron, molybdenum, phosphorus, and the heavy metal tin, a nitrous oxide–acetylene burner and flame were used. Approximately 1-g samples were weighed

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