(200 mL) for 6 hr under anhydrous conditions. The reaction mixture was cooled and the solid matter (6.5 g, white powder, sulfur test positive, no lather with water) was filtered off and washed with 3 x 30 mL of benzene. The benzene was distilled off and the crude residue (7.2 g) was chromatographed on silica gel (1:30) with benzene and ether as eluants. The benzene eluate (6.1 g), upon chilling with petroleum ether, gave santalbic acid (4.7 g). The ether eluate (0.8 g) was a reddish-yellow liquid, found to be a mixture of mono- and dimethyl phthalate, as confirmed by Co-TLC and Co-IR.

RESULTS AND DISCUSSION

Methylation of phthalic anhydride using the sodium santalbate-dimethyl sulfate complex in benzene medium and yielding a mixture of dimethyl and monomethyl phthalate, suggests that the dimethyl sulfate, which is physically adsorbed in the complex with sodium santalbate, is released by heat. Further evidence regarding the physical adsorption of dimethyl sulfate on sodium santalbate is provided by the IR spectrum of the complex, with the characteristic frequencies for $-\overset{\overset{O}{\|}}{C}-O^-$ at 1560 cm^{-1}, S=O at 1250-1220 cm^{-1}, C-O at 1000 cm^{-1} and S-O at 770 cm^{-1}. This paper is only intended to report that the normal reaction product between the salt of an acetylenic acid and a methylating reagent like dimethyl sulfate is not the methyl ester, but a molecular inclusion complex, hitherto unknown as far as sodium santalbate and dimethyl sulfate are concerned, and that the complex has a demonstrative cleansing property.

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Extraction of Alkaloids and Oil from Bitter Lupin Seed

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ABSTRACT

Quinolizidine alkaloids and the oil were recovered from seeds of bitter lupin, *Lupinus mutabilis*, by 2 extractions using hexane as the only organic solvent. Ground and flaked lupin seed was extracted first with hexane, which recovers the oil and those alkaloids that occur as free bases. Subsequently, the hexane-insoluble salts of the alkaloids retained in the defatted flakes were converted into hexane-soluble free bases by treatment with aq sodium carbonate or ammonium hydroxide and removed by another extraction with hexane. A low-alkaloid proteinaceous meal was obtained with practically no loss of protein. The alkaloids dissolved in the oil were completely recovered by extraction with aq hydrochloric acid.

INTRODUCTION

The lupin, a leguminous plant, has great potential as an oilseed crop in regions having a temperate climate (1,2). The seeds of bitter lupin, *Lupinus mutabilis*, a variety widely cultivated in the Andean regions of South America, constitute a rich source of edible oil and protein (3). The use of products from bitter lupin seed in food and animal feed is restricted, largely due to the occurrence of toxic quinolizidine alkaloids (1). Breeding of *L. mutabilis* for the production of low-alkaloid, "sweet" lupin seed is still at an experimental stage (4).

Currently, the bitter lupin seed is processed in a manner similar to other oilseeds (2), i.e., the crushed and flaked seeds are extracted with hexane to yield the oil and a minor portion of the alkaloids. An edible oil is then obtained by refining, which leads to a complete removal of the alkaloids. The defatted lupin meal containing a major portion of the alkaloids must be "detoxified" prior to use in food and feed.

Blaicher et al. have shown recently that protein concentrates, virtually devoid of alkaloids, can be prepared from hexane-defatted meal of bitter lupin seed by extraction with aq alcohols (5). Although such protein concentrates should be eminently suitable for dietary supplement of an undernourished population, the production of these concentrates involves rather sophisticated technology that deviates considerably from the conventional processing of oilseeds. Thus, the use of 2 different solvents, i.e., hexane for defatting and aq alcohol for the removal of alkaloids and soluble carbohydrates, requires additional, elaborate equipment for solvent recovery.

In this work, we have explored the possibility of recovering alkaloids and oil from bitter lupin seed by extraction with hexane as the sole organic solvent. Our aim was to find a process that can be easily adapted to the existing technology of oilseed extraction to produce an edible oil and a low-alkaloid proteinaceous meal.

EXPERIMENTAL PROCEDURES

Material

Seeds of bitter lupin, grown in Chile, were crushed and flaked in a pilot plant as described elsewhere (5,6).

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Analytical Methods

Standard AOCS methods were used for the determination of moisture (7) total nitrogen (8) and fat (9). Protein contents were calculated as (total nitrogen-alkaloid nitrogen) x 6.25. Total quinolizidine alkaloids were determined according to a procedure described previously (5,10).

Extraction Procedures

The flakes of bitter lupin seed were defatted by extraction with hexane in a Soxhlet apparatus at 50 C for 12 hr. For the removal of alkaloids, the defatted lupin flakes were treated as described next.

Portions of the defatted lupin flakes, 10 g each, were taken in glass-stoppered Erlenmeyer flasks and mixed uniformly with definite amounts (up to 3.0 mL/g flakes) of sodium carbonate or ammonium hydroxide at various concentrations (up to 30%). Definite volumes (5-25 mL/g flakes) of hexane were then added and the mixture was shaken for different periods (5-20 min) in a water bath at various temperatures (30-60 C) using a gyratory shaker (Type WTR-1, Infors, AG, CH-4015 Basel). Thereafter, the extract was separated from the flakes by suction on a heated Buchner funnel, and the extracted flakes were dried and analyzed.

Alternatively, the defatted lupin flakes, after mixing with the aq base, were consecutively extracted with hexane as just described. After each extraction, the flakes were transferred back to the Erlenmeyer flask and reextracted twice with fresh solvent.

In order to minimize solvent requirement, the defatted lupin flakes, after mixing with the aqueous base, were extracted with hexane by a countercurrent procedure. Four-stage countercurrent extractions of 40 g of flakes, divided into 10-g batches, were done as already described according to the scheme given elsewhere (11).

Large-scale extractions of the defatted lupin flakes were done in a batch extractor (5). Batches of 2 kg of flakes were mixed with 15% sodium carbonate (1 mL/g flakes) and extracted with 16 L of circulating hexane (200 L/hr) for 1 hr at 60 C. After separation of the extract, the extractions were repeated 2 more times with fresh hexane.

In order to recover the alkaloids dissolved in the oil, the latter was extracted 3 times in a separatory funnel with 2% its volume of 5% hydrochloric acid. The fat was then washed several times with water until neutral, and its alkaloid content was determined titrimetrically (10).

RESULTS AND DISCUSSION

In plant materials, alkaloids are known to occur partly as free bases and partly as salts that are insoluble in most organic solvents. A common practice for the isolation of alkaloids from plant sources, prior to their characterization, consists of treatment with a base that converts such salts into "free" alkaloids, which, being soluble in organic solvents, can be easily recovered by extraction (12). In this study, we have explored essentially a similar approach as a possible technological process for the recovery of alkaloids and oil from the bitter lupin seed. Thus, the flakes prepared from bitter lupin seed were extracted with hexane in order to recover the oil and the alkaloids that occurred as free bases. Subsequently, the defatted flakes containing the alkaloids bound as salts were treated with an aqueous base in order to liberate the alkaloids which were then recovered by another extraction with hexane.

The bitter lupin seeds used in this study contained, on a moisture-free basis, 42.5% protein, 19.8% oil and 4.2% alkaloids. Extracting the flaked seeds with hexane yielded defatted flakes containing 53.4% protein, 1.1% oil and 3.2% alkaloids. About 40% of the alkaloids was recovered together with the oil by extraction with hexane. These alkaloids apparently occurred in the flakes as free bases which are soluble in hexane.

The defatted lupin flakes were treated with varying proportions of an aqueous base, such as sodium carbonate or ammonium hydroxide, and the alkaloids were subsequently recovered by extraction with hexane. The effects of various parameters on the recovery of alkaloids are summarized in Tables I and II.

It is quite evident from the data in Table I that the treatment with sodium carbonate facilitated the recovery of as much as 70% of the remaining alkaloids from the defatted lupin flakes by a single extraction with hexane. As to be expected, increasing the temperature, time and the amount of hexane increases the recovery of alkaloids. Moreover, the concentration and the amount of aq sodium carbonate had pronounced effects on the recovery of alkaloids. Under the conditions used, the use of 1 mL 15% sodium carbonate/g defatted flakes permitted the best recovery of alkaloids.

Essentially similar results were obtained when defatted flakes were treated with aq ammonium hydroxide, instead of sodium carbonate, prior to extraction of the alkaloids with hexane (Table II).

The recovery of alkaloids ranged 80-90% when the defatted lupin flakes, treated with aq sodium carbonate or ammonium hydroxide, were extracted consecutively 3 times or according to the countercurrent procedure (Table III). Advantages of the countercurrent procedure are obvious in view of solvent economy.

In all the foregoing experiments, the hexane extracts after the recovery of oil and alkaloids were free of proteins. Thus, practically none of the proteins contained in the bitter lupin seed were lost by these processes.

The oil recovered by extraction with hexane contains the free alkaloids. Treatment of this oil with aq hydrochloric acid converted these alkaloids into water-soluble salts that were easily removed by repeated extractions with water. The oil resulting from this treatment was virtually free of alkaloids, even prior to conventional refining.

Treatment with an inorganic base for the removal of undesirable constituents from oilseed meals has been proposed, e.g., for the removal of aflatoxins from cottonseed and peanut meals (13). Our studies show that, in a similar manner, most of the undesirable quinolizidine alkaloids can be efficiently removed from bitter lupin seed by a simple process that can be conducted in a conventional oil extraction plant. The resulting lupin meal containing about 55% protein has a slightly higher level of alkaloids (0.3-0.4%) compared to the lupin protein concentrates described earlier (5) or the sweet lupines (14). It is conceivable that the process described can be further simplified and the recovery of alkaloids improved if the crushed lupin seed is treated outright with a base and then extracted with hexane to recover the oil and most of the alkaloids simultaneously by a single extraction.

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TABLE I

Recovery of Alkaloids from Defatted Lupin Flakes by Treatment with Sodium Carbonate and a Single Extraction with Hexane

Treatment with sodium carbonate solution		Extraction with hexane			Alkaloids recovered (%)
Amount added (mL/g flakes)	Concentration (%)	Amount of hexane (mL/g flakes)	Time (min)	Temperature (C)	
				30	34
1.0	15.0	15	10	40	45
				50	53
				60	63
			5		58
1.0	15.0	15	10	60	62
			15		66
			20		70
		5			46
		10			59
1.0	15.0	15	10	60	63
		20			64
		25			73
	0.0				1
	7.5				53
1.0	15.0	15	10	60	62
	22.5				60
	30.0				54
0.0					1
0.5					29
1.0	15.0	15	10	60	62
2.0					47
3.0					38

TABLE II

Recovery of Alkaloids from Defatted Lupin Flakes by Treatment with Ammonium Hydroxide and a Single Extraction with Hexane

Treatment with ammonium hydroxide solution		Extraction with hexane			Alkaloids recovered (%)
Amount added (mL/g flakes)	Concentration (%)	Amount of hexane (mL/g flakes)	Time (min)	Temperature (C)	
			5		58
1.0	7.5	15	10	60	63
			15		67
			20		69
	0.0				1
	7.5				63
1.0	15.0	15	10	60	67
	22.5				67
	30.0				66
0.0					1
0.25					39
0.5	7.5	15	10	60	60
1.0					63
1.5					55

TABLE III

Recovery of Alkaloids from Defatted Lupin Flakes by Treatment with a Base and Consecutive or Coutercurrent Extractions with Hexane

Treatment	Batch size (g)	Extractions with hexane[a]		Alkaloids recovered (%)
		Amount of hexane (mL/g flakes)	Mode of extraction	
	10	45	Consecutive	85
1 mL 15% Na_2CO_3 /	2000	24	Consecutive	88
g flakes	40	15	Countercurrent	86
1 mL 7.5% NH_4OH/	10	45	Consecutive	88
g flakes	40	15	Countercurrent	79

[a]All extractions except for the 2000-g batch were done at 60 C for 15 min; extractions of the 2000-g batch were done at 60 C for 1 hr with circulating hexane (200 L/hr).

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Factors Affecting Slip Melting Point of Palm Oil Products

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ABSTRACT

The effect of different factors affecting the slip melting point of palm oil has been evaluated. The most important factor appears to be the difference in tempering temperatures. The influence of different tempering temperatures on slip point values is, however, dependent on the nature of the sample. For hydrogenated oils and for some high-melting palm stearins, tempering has no effect. For palm oil and palm olein, higher melting points were obtained when tempering at the higher temperatures in the range of 4-15 C. For some soft stearins, however, lower melting points were obtained at the higher tempering temperatures. These effects are investigated with differential scanning calorimetry and an explanation is offered, based on phase diagrams. A secondary effect on the slip melting point was the height of fat in the capillary tube. Effects of using different methods of determination are also shown. Collaborative trials on a standard testing procedure, AOCS Cc3-25, revealed the inadequacy for palm oil of the temperature range of 4-10 C specified in the procedure and its fractions. Strict adherence to a fixed tempering temperature produced better precision and reproducibility among laboratories. Tempering at 10 ± 1 C is recommended.

INTRODUCTION

A number of standard procedures is available for the determination of the slip melting point of fats, e.g., AOCS Cc 3-25 and Cc 1-25 (1), British Standard 684 (2), Indian Standard 548 (3) and as described by Cocks and Van Rede (4). The methods differ in important details as shown in Table I.

For some fats, pretreatment has an important effect on the value obtained, particularly when the fat shows polymorphic behavior. For example, the British Standard method 2 (2) recommends a special procedure for such fats, and it is well known that an elaborate procedure must be followed in the case of cocoa butter. The melting point of fats is an important item of many specifications used in trade and, in some countries, is an element of the legal definition of food products. Widespread conformity in methods and in the results obtained is therefore of importance to the oils and fats industry worldwide. This study was prompted by the large interlaboratory variations found in ring tests of the AOCS method Cc 3-25 (1) when applied to palm oil products.

EXPERIMENTAL PROCEDURES

Samples

Commercial samples of refined palm oil, palm olein, hydrogenated palm oil (IV 44.6), palm stearin of different iodine values, hydrogenated rapeseed (IV 79.9) and soybean oils (IV 75.8) and of Indian vanaspati were examined. The palm olein and stearin samples were obtained from the fractionation of palm oil on the commercial scale.

TABLE I

Differences Among Slip Point Methods

Methods	Internal diam. of capillary tubes	Tempering procedure	
		Initial treatment	Further treatment
Cocks and Van Rede	0.9-1.1 mm	Chill against ice until solid	Hold at -10 C for 5 min
AOCS Cc 3-25	1 mm	Chill against ice until solid	Hold at 4-10 C for 16 hr
British Standard 684			
Method 1	1.1-1.3 mm	Cool to 15 C	Hold at 15-17 C for 16 hr
Method 2	1.1-1.3 mm	Cool with stirring until a paste is formed Fill capillary tube with paste.	Hold at 15-19 C for at least 24 hr
Indian Standard 9.1	0.8-1.1 mm	Chill against ice until solid	Hold at 4-10 C for 1 hr