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Application of colorimetric assay for the determination of cyanide in deoiled jojoba meal

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APPLICATION OF COLORIMETRIC ASSAY FOR
THE DETERMINATION OF CYANIDE IN
DEOILED JOJOBA MEAL

A Thesis
Presented to
The Faculty of the Department of Nutrition and Food
Science
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in Nutritional Science

by
Joselyn Jenniffer Allen
May 1997

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ABSTRACT
APPLICATION OF COLORIMETRIC ASSAY FOR
THE DETERMINATION OF CYANIDE IN
DEOILED JOJOBA MEAL

by Joselyn Jenniffer Allen

The colorimetric method developed by Lambert et al. (1975) and modified by Haskins et al. (1988) for the determination of cyanide was applied in the determination of total cyanide in deoiled jojoba meal. Patel et al. (1992) preliminary investigation found this method can be utilized in jojoba meal. The procedure involves grinding the dry deoiled jojoba meal, extracting the cyanogenic glycoside simmondsin with water, combining alkali and heat of the extract and colorimetric determination of cyanide. Applicability of the procedure was tested using pure simmondsin. Since our preliminary data showed that results obtained from jojoba extracts are influenced by the number of extractions, amount of NaOH and heating time, these parameters were optimized. Results obtained showed a concentration of 1.6 milliequivalent NaOH to be the optimal concentration of alkali in the liberation of cyanide in deoiled jojoba meal. Maximum cyanide levels were attained after a 3 minute heating in a boiling water bath. To obtain total cyanide content, at least three extractions of a single sample of jojoba meal is required. Based on a standard curve of KCN (100 ppm) solution, total cyanide content of deoiled jojoba meal determined by this method ranged from 2282 to 3250 ppm. Researchers studying the utilization of jojoba meal for a livestock ingredient can apply this simple and reliable method to the determination of cyanide in detoxified jojoba meal.

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PREFACE

The following thesis is written in publication style. The second chapter is written in journal format and will be submitted to the The Journal of Agricultural and Food Chemistry. Chapter I and III are written according to the guidelines in the Publication Manual of the American Psychological Association, 4th. edition, 1995.

Table of Contents

	Page
List of Figures.....	x
List of Tables.....	xii
 CHAPTER	
1. INTRODUCTION AND REVIEW OF LITERATURE.....	1
Introduction.....	1
Objectives.....	4
Significance of Study.....	4
Review of Literature.....	5
Jojoba.....	5
Cyanogenic Glycosides.....	7
Detoxification of Jojoba Meal.....	13
Determination of Cyanide.....	14
2 JOURNAL ARTICLE.....	19
Authors' Title Page.....	20
Abstract.....	21
Introduction.....	22
Materials and Methods.....	24
Reagents.....	24
Standards for Calibration.....	24
Sample Preparation.....	24
Maximum Wavelength.....	24
Number of Extractions.....	25
Determination of Optimal Amount NaOH on the Formation of Cyanide in Pure Simmondsin.....	25

	Determination of Optimal Heating Time on the Formation of Cyanide in Deoiled Jojoba Meal.....	26
	Colorimetric Procedure.....	26
	Results and Discussion.....	27
	Effect of Multiple Extractions on the Determination of Cyanide in Jojoba Meal.....	27
	Effect of Heat Treatment on the Liberation of Cyanide in Pure Simmondsin and Deoiled Jojoba Meal.....	27
	Effect of NaOH Concentration on Cyanide Formation in Pure Simmondsin.....	29
	Effect of Heating Time on Formation of Cyanide in Pure Simmondsin.....	29
	Total Cyanide Liberated from Pure Simmondsin.....	29
	Effect of NaOH Concentration on Cyanide Formation in Deoiled Jojoba Meal.....	30
	Effect of Heating Time on Cyanide Formation in Deoiled Jojoba Meal.....	30
	Total Cyanide Liberated from Deoiled Jojoba Meal.....	31
	Correlation of Cyanide Concentration to Varying Amount of Jojoba Meal.....	31
	Conclusion.....	32
	Acknowledgement.....	32
	Literature Cited.....	45
3	SUMMARY AND RECOMMENDATIONS.....	47
	Summary.....	47
	Recommendations.....	49

References.....	50
-----------------	----

List of Figures

Figure	Page
1. The structure of simmondsin and simmondsin ferulates.....	8
2. The structure of cyanoglycosides, and their metabolism to release cyanide.....	9
3. The structures of major cyanoglycosides.....	10
4. Absorbance of the summation of three extractions from single sample of jojoba meal treated with 0.9 milliequivalents NaOH after heating for 3 minutes.....	33
5. Absorbance spectra of the color of cyanide in varying aliquotes of jojoba meal treated with 1.6 milliequivalents NaOH: 0.10 ml extract (A), 0.08 ml extract (B), 0.06 ml extract (C), 0.04 ml extract (D), 0.02 ml extract (E) after 3 minute boiling time and 0.10 ml extract without heat treatment (F).....	34
6. The effect of NaOH concentration on the liberation of cyanide in pure simmondsin at 10 minute boiling time.....	35
7. The effect of heating time on the liberation of cyanide in pure simmondsin.....	36
8. The effect of NaOH concentration on the development of color in deoiled jojoba meal.....	37
9. The effect of heating time measured in second intervals on the formation of cyanide in jojoba meal treated with 0.9 milliequivalents NaOH.....	38
10. The effect of heating time measured in minute intervals on the formation of cyanide in jojoba meal treated with 0.9 milliequivalents NaOH.....	39

11.	The effect of heating time measured in minute intervals on the formation of cyanide in 3 combined extractions of jojoba meal treated with 0.9 milliequivalents NaOH.....	40
12.	The correlation of cyanide liberated from varying amounts of jojoba meal treated with 1.6 milliequivalents NaOH and heated for 3 minutes.....	41

List of Tables

Table	Page
I. Total cyanide liberated from 1.0 mg pure simmondsin heated at varying time and treated with 1.8 milliequivalents NaOH.....	42
II. Total cyanide liberated from 1.0 mg pure simmondsin treated with varying amounts of NaOH and heated for 40 minutes.....	43
III. Total cyanide liberated from 3 extractions of jojoba meal treated with 1.6 milliequivalents NaOH after 3 minutes heating time.....	44

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Jojoba (*Simmondsia chinensis*), is a perennial shrub indigenous to arid land of the southwestern United States and the Sonoran desert of western Mexico. It is becoming more widely cultivated in certain hot countries such as Africa, Israel, South America, Australia, and the Mediterranean for its oil-containing nuts. Commercial propagation of jojoba began in the early 1970's, when the United States government banned the importation of sperm whale oil to protect the endangered species (NAS, 1975). Jojoba oil has replaced the use of sperm whale oil in many industrial and commercial applications because of its similar physical and chemical properties.

Jojoba oil represents approximately 50% of the whole seed and is composed primarily of a liquid wax. Though jojoba oil contains trace amounts of triacylglycerol (Busson-Breysse et al., 1994), it is mainly composed of straight chain mono-esters of long chain mon-ethylenic alcohols and fatty acids which makes it an appropriate replacement for sperm whale oil (Wisniak, 1977; NAS, 1975, 1977).

The residue remaining after solvent and mechanical extraction of jojoba oil is the meal. Because jojoba meal contains approximately 20-34% protein, studies have focused on its utilization as a potential feed ingredient for livestock. Presently, jojoba meal has no commercial uses because studies have shown adverse effects in feed intake, feed efficiency, growth and overall nutritional status in chicken, sheep, cattle and laboratory test animals when fed rations containing 4% to 10% deoiled jojoba meal (Both et al., 1974; Verbiscar

et al., 1978, 1980, 1981; Amouts et al., 1993; Cokelaere et al., 1992). A closely related group of compounds called cyanogenic glycosides have been isolated from jojoba meal and are thought to be responsible for these adverse effects. Simmondsin and simmondsin 2' ferulate, the major cyanoglycosides, and simmondsin 3'-trans-ferulate (Van Boven et al., 1994) have been identified to produce a powerful food intake inhibition effect (Both et al., 1974; Cokelaere et al., 1992). The emaciation effect of jojoba meal was attributed not only to reduction of food intake but also to a supplementary toxicological influence of simmondsin and its analogues (Booth et al., 1974).

Cyanogenic glycosides have been identified in over 2,000 species of higher plants (Majak, 1992). Though widely distributed in the plant kingdom, there is no simple pattern of glycoside distribution. Prunasin and linamarin occur in a number of different families, while amygdalin only occur in the Rosaceae family (Majak, 1992). Linamarin, amygdalin, prunasin and dhurrin are the major cyanogenic glycosides important to human and animal nutrition. Linamarin is present in linseed (flaxseed), cassava and lima beans. Amygdalin and prunasin are present in service berries, mountain mahogany, saskaton and kernels of cherries, almonds, apricots, peaches, apples and plums (Conn, 1969; Poulton & Chun Ping, 1994). Dhurrin is present in grain sorghums, forage sorghum leaves and sudangrass (Haskins et al., 1988). In certain areas of the world, such as the tropics where sorghum, lima beans and especially cassava are major staples in the diet, cyanogenic glycoside have a great nutritional impact on the human diet (Majak, 1992). Cyanogenic glycosides are toxic because they release a lethal metabolite, hydrocyanic acid (HCN), upon hydrolysis. Cyanide is extremely toxic because it inhibits the cytochrome

oxidase in the electron transport chain, thereby blocking aerobic cellular respiration and preventing the utilization of oxygen (Majak, 1992).

Upon plant tissue disruption, cyanogenic glycosides are hydrolyzed. This is due to the contact between the cyanogenic substrates and the plants' endogenous hydrolytic enzymes, ie. beta-glucosidases and hydroxynitrile lyases. The substrate and enzymes are found in separate cell compartments of the plant and are not hydrolyzed to liberate HCN unless otherwise disrupted or crushed (Gruhnert, Biehl, & Selmar, 1994). Typically, the most common form of processing cassava (*Manihot esculenta*) for consumption is crushing the tuber and soaking in water for many days. This ensures both enzymatic and water hydrolysis to facilitate detoxification of cyanide.

The toxicity of released cyanide in edible plants containing cyanoglycosides is well recognized. Cyanogenic plants release HCN as a product of disintegration. Analytical methods for the determination of cyanide have been utilized to evaluate toxicity in cyanogenic plants. Various instrumental techniques have been applied to the determination of cyanoglycosides including spectrophotometry, potentiometry, liquid chromatography, nuclear magnetic resonance spectrometry, chemical ionization mass spectrometry, and gas chromatography (Kupchella & Syty, 1984). The determination of cyanide in cyanoglycoside containing plants has been the main focus of analytical methods due to its known toxicity. However, there is conflicting data on the cause of toxicity in jojoba meal. Williams (1980) attributed toxicity of simmondsin to the formation of cyanides during metabolism of simmondsin in the rat. While Cokeleare et al. (1992) observed no elevated concentrations of cyanide or thiocyanate in the blood when given orally to rats,

concluding that there is no indication for the liberation of HCN during the metabolism of simmondsin in the rat. Nevertheless, the toxicity of simmondsin and its analogues have never been fully proven nor have the physiological mechanisms by which it inhibits food intake (Van Boven et al., 1993). It is known that jojoba meal contains the lethal metabolite cyanide, though its mechanism of degradation is still unknown. The determination of cyanide in jojoba meal may assist to elucidate the metabolic conversion during the hydrolysis of simmondsin and its analogues.

Objective

The objective of this study was to establish the optimal conditions for the colorimetric procedure of Lambert et al. (1975) as modified by Haskins et al., (1988), applied to the determination of cyanide in deoiled jojoba meal. The total cyanide content of deoiled jojoba meal was determined using the optimal parameters obtained for this method.

Significance of the Study

Commercial jojoba oil processors have long been interested in potential uses for the large amount of by-product currently being discarded. Researchers are presently studying the effects of jojoba meal supplementation on test animals for applications in the livestock feed industry, however, direct determination of simmondsin via PHLOX is time consuming and costly. No studies on the determination of cyanide in deoiled jojoba meal have been conducted. For a greater understanding of the mechanism of toxicity of jojoba meal, the need for a rapid and reliable method for cyanide analysis is evident.

Review of Literature

Jojoba

Jojoba (*Simmondsia chinensis*) is a natural desert plant native to the Sonoran Desert of northwestern Mexico and neighboring areas in Arizona and southern California (NAS, 1975). This perennial shrub, recognized by its flat, gray-green, leathery leaves, grows to a height range of 2-4 feet in areas of low rainfall, and as tall as 15 feet in areas of high rainfall (NAS, 1977). Because of its deep and extensive root system which traps all available soil moisture, it is resistant to drought and grows in areas where most other plants wither and die (NAS, 1977). Jojoba grows best in well-drained, coarse desert soils with a mixture of gravels and clays (NAS, 1975). Jojoba has unisexual flowers which occur on separate plants; however, the female plant bears the fruit which contains the seeds (Wisniak, 1977).

Commercial interest in jojoba began in December, 1970, after provisions made under the Endangered Species Conservation Act banned the importation of oil, meat and other products obtained from whales (NAS, 1975). For many decades the only source of liquid wax was from sperm whale and the demand from industry, the military, and space programs was great. Jojoba cultivation grew to a multi-million dollar industry with up to 11,000 to 18,000 tons of dry seeds being planted in the United States and Mexico each year.

Jojoba seeds contain approximately 40-65% lipids which constitute a liquid wax and are composed of a mixture of fatty acids and fatty alcohol esters. The fatty acid composition is 76.7% eicos-11-enoic acid (C20:1) and 12.1% contained docos-13-enoic (C22:1). The fatty alcohol ester composition is 45.4% eicos-11-enol and 46.4% docos-13-enol with 6.5% tetracos-15-enol. Fourteen

distinct esters have been identified. Jojoba oil also contains some triacylglycerols, free fatty esters and seven newly identified sterols as minor constituents (Busson-Breysse, et al., 1994). When jojoba oil is hydrogenated, it forms a dense solid of white crystalline wax which resembles spermaceti, carnauba wax, and beeswax in chemical and physical properties (NAS, 1977). Its applications include uses in cosmetics due to its shelf stability and is used in pharmaceutical preparations as a stabilizer. It does not undergo oxidative or hydrolytic rancidity. Jojoba wax is not hydrolyzed by digestive lipases, enabling its use as a noncaloric "fat" for diabetics. Because of its low and constant viscosity over a large temperature range, it is often used as a high-pressure lubricant or a neutral oil (Busson-Breysse et al., 1994).

Once the oil from the jojoba seeds has been extracted, a residue or meal remains. This by-product constitutes approximately 50% of the whole seed. Defatted jojoba meal contains approximately 26 to 30% protein, 12% simple sugars, and 16% complex carbohydrates in the form of starch (Wisniak, 1977). Of the essential amino acids studied, the content of lysine is high; however, the methionine content is low (NAS, 1975). Because of the relatively high amount of protein content in jojoba meal, much interest has been given for its use as a potential feed ingredient for livestock (NAS, 1975, 1977; Verbiscar et al., 1980).

Jojoba meal is unsuitable for livestock feeding because it contains toxic or antinutritive compounds that produce adverse effects on total food intake, food digestibility and growth rate in rats, chickens and lambs (Manos et al., 1986; Arnouts et al., 1992; Cokelaere et al., 1992). Booth et al. (1974) attributed these adverse effects to the presence of the cyanoglycosides simmondsin and simmondsin 2' ferulate. The presence of simmondsin and simmondsin 2'

ferulate in jojoba meal has been shown to be 3.6 to 6.0 percent and 0.35 to 2.0 percent, respectively. Elliger et al. (1974b) described two simmondsin analogues as two monodemethylsimmondsins. In 1988, Abbot et al., described the isolation of the compounds and characterized them as demethylsimmondsin (DMS) and didemethylsimmondsin (DDMS); however, they did not provide the stereochemistry nor the details of structure analysis. Van Boven et al., (1994), isolated and structurally identified a new simmondsin analogue in jojoba meal called simmondsin 3'-trans-ferulate. In addition, Van Boven et al., (1994b), isolated the exact structure and stereochemistry of simmondsin as [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl beta-D-glucoside] and the major simmondsin analogues as 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl beta-glucoside and 2-(cyanomethylene)-3,4,5-trihydroxycyclohexyl beta-glucoside as well as simmondsin 2' ferulate and simmondsin 3' trans-ferulate (Figure 1).

Cyanogenic Glycosides

Although found in trace amounts, cyanide is almost ubiquitous in the animal kingdom. High levels of cyanide, in the form of cyanoglycosides can be found in certain forage species, root crops, grains, pulses and fruit kernels (Aleator, 1993). Cyanoglycosides can be found in over 2,000 species of higher plants. Most noted for cyanogenesis are the families of the Rosaceae, Leguminosae, Gramineae and Euphorbiaceae which compose of almost 25% of the plants capable of cyanide production (Majak, 1992). The cyanoglycosides found in the most concentrated levels include linamarin (present in cassava and flax seed) and phaseolunatin (present in lima bean or other pulses). Amygdalin is present in the *Rosaceae* species, including cherries, mountain mahogany,

Figure 1. The structure of simmondsin and simmondsin ferulates

service berries and is also found in the kernels of almonds, apricots, peaches and apples. Dhurrin and lotaustralin are commonly found in sorghum and other grasses used as fodder (Aletor, V. A., 1993). It is generally believed that the toxic effect of cyanoglycosides is attributed to the release of hydrocyanic acid (HCN) upon hydrolysis of the nitrile containing aglycone. Unstable cyanohydrins (alpha-hydroxynitriles) are the aglycones resulting from the hydrolysis of cyanoglycosides. They rapidly dissociate at physiological pH to yield hydrocyanic acid (HCN) and either an aldehyde or a ketone (Figure 2) (Bennett & Wallsgrove, 1994). The hydrolysis of cyanohydrin (hydroxynitrile) is stable in acidic conditions, but very reactive in basic conditions (Selmar et al., 1978). Hydrolytic enzymes are compartmentalized in the tissue level of the plant as opposed to the subcellular level (Poulton & Li, 1994). Once the tissue is crushed or disintegrated, the enzymes react with the cyanoglycoside to release HCN. The cyanogenic glycoside, prunasin yields benzaldehyde, glucose and cyanide and linamarin yields glucose, cyanide and acetone.

Cyanide, either in synthetic inorganic form such as KCN or NaCN, or in naturally occurring organic form such as cyanoglycosides, acts as a potent

Figure 2. The structure of cyanoglycosides, and their metabolism to release cyanide.

specific and non-specific inhibitor of many enzyme-catalyzed reactions in the body. Cyanide is known as a potent respiratory inhibitor because it impairs the transfer of electrons released during the oxidative phosphorylation to molecular oxygen. Cyanide blocks cytochrome oxidase, the terminal catalyst of aerobic organisms in the electron transport chain, thereby preventing the utilization of cellular oxygen (Conn, 1979).

In certain areas of the world, such as the tropics where sorghum, lima beans and especially cassava are major staples in the diet, cyanogenic glycoside have a great nutritional impact on the human diet (Majak, 1992). The structure of these compounds are shown in Figure 3. Cyanogenic glycosides such as amygdalin and prunasin are found in the seeds of plants of the apple, cherry, almond, and peach which all belong to the Rosaceae family. Prunasin is structurally similar to amygdalin in that it contains one less glucose unit and is found in all parts of the plant, where amygdalin is only found in the seeds (Miller, 1973, Kupchella & Syty, 1984). When the seeds of the Rosaceae mature, amygdalin content increases while prunasin content decreases

Figure 3. The structure of major cyanoglycosides.

indicating that prunasin may be a precursor to amygdalin in the seed (Mizutani et al., 1979). When the seeds of the Rosaceae are crushed, the enzyme emulsin, naturally found in the seeds of the Rosaceae, influence the hydrolysis of the cyanoglycosides yielding hydrocyanic acid, benzaldehyde and glucose (Kupchella & Syty, 1984). The presence of endogenous enzymes in jojoba meal have not been studied. Compared with cyanoglycosides mentioned here, simmondsin and its analogues possess a more complex structure which is resistant to typical hydrolysis. In addition, the hydrolytic products of the cyanoglycosides found in jojoba meal are unknown.

Van Boven et al., 1993, isolated highly purified simmondsin to be utilized for larger animal studies. The toxicity of simmondsin and other related cyanomethylenecyclohexyl glycosides as well as the physiological mechanisms by which simmondsin inhibit food intake have never been fully understood or proven. Williams, 1980, studied the cyanide and thiocyanate levels in the blood of mice which were administered simmondsin. Williams described the toxicity of jojoba meal to the presence of cyanides; specifically, the liberation of cyanide

from simmondsin to form hydrocyanic acid. However, he was unable to find a specific histopathological effects on the internal organs in the test animals attributed to simmondsin. In another study with similar findings, lambs were given feed supplemented with five or ten percent jojoba meal for 80 days. The animals were leaner than their controls, yet showed no morbid symptoms. The researchers found no organ damage and concluded that the cyanoglycosides in jojoba are detoxified by microorganisms in the gut of ruminants (Manos et al., 1986)

Cokelaere et al. (1992) conducted a study to investigate possible toxicological influences of simmondsin after subacute administration in the rat. A 5-day administration of 250 mg of simmondsin/kg of body weight did not produce any toxicological effects on the liver, pancreas, kidneys, intestine, stomach, testes, and seminal vesicles. In addition, concentrations of CN- and SCN- in the blood were not found to be elevated. The researchers found evidence of food intake inhibition and a weight-reducing effect of simmondsin but concluded that this effect was not caused by the production of HCN. There were no indications for the liberation of cyanide during the metabolism of simmondsin. Cokelaere et al. (1992a) conducted another study to determine the influence of pure simmondsin on the food intake of rats. The results indicated an inhibitory effect on food intake, however, no cyanides were detected following metabolism of simmondsin. Therefore, the toxicity of simmondsin was thought to be a direct result of the food inhibition effect as opposed to simmondsin metabolites produced in the body.

Arnounts et al. (1993) studied the ability of jojoba meal to inhibit feed intake of broiler breeder pullets to limit weight gain as recommended by the

breeder company. The pullets with diets supplemented with jojoba meal produced a reduction of growth rate with increased plasma growth hormone and thyroxine and with decreased plasma insulin-like growth factor-I and triiodothyronine concentrations compared with pullets without jojoba meal supplementation. The researchers concluded that the growth retardation caused by jojoba meal supplementation was provoked by an inhibition of appetite linked with simmondsin content as well as other antinutritional compounds found in jojoba meal affecting digestibility such as polyphenols, phytic acid and trypsin inhibitors.

Other studies in rats have demonstrated that simultaneously administering a specific cholecystokinin receptor antagonist, devazepide, with jojoba meal reverses the food inhibition effect of simmondsin (Cokelaere et al., 1995a). The authors suggest that simmondsin exerts the food inhibition effect by inducing satiation via the cholecystokinin system.

The paraenteral LD₅₀ in rats is several times higher than the oral LD₅₀, therefore, researchers hypothesized that during the ingestion of cyanoglycosides by rats, a small amount of hydrolysis occurs due to the normal intestinal microflora but most of it is excreted unchanged in the urine (Oke, 1981). Some authors have suggested that intraperitoneally injected simmondsin compared with orally administered simmondsin apparently does not reduce food intake or body weight over a 24 hour period because bacterial metabolites of simmondsin are absorbed into the bloodstream (Booth et al., 1974; Verbiscar et al., 1980). However, Flo et al. (1997) conducted a study to determine the absorption and excretion of simmondsin by different administration routes in rats and found conflicting data. The researchers found

that rats administered simmondsin via intraperitoneal injection did in fact produce food intake inhibition over a period of 24 hours when compared to intragastric intubated rats and rats fed simmondsin mixed with food, respectively. According to the researchers, the results indicate that simmondsin passes from the blood into the proximal gut where it seems to be necessary for the induction of anorexia.

The mechanism involved in the liberation of cyanide in most cyanoglycosides is well understood. When the nitrile containing aglycone is hydrolyzed an unstable cyanohydrin is produced. At physiological pH, the cyanohydrin or hydroxynitrile undergoes dissociation to yield glucose, hydrocyanic acid (HCN) and either a ketone or aldehyde. However, the mechanism for the liberation of cyanide in the cyanoglycoside, simmondsin, is yet unknown.

Detoxification of Jojoba Meal

Several methods for the detoxification of jojoba meal have been reported (Elliger et al., 1975; Banigan & Verbiscar et al., 1980, 1981). Verbiscar et al. (1980) investigated a number of solvent extraction, heat, and chemical methods to detoxify jojoba. A chemical process using ammonium hydroxide and hydrogen peroxide was found to be the most effective chemical method, reducing simmondsin levels to 0.19 percent and simmondsin 2' ferulate levels to 0.08 percent or less. (Banigan & Verbiscar, 1980). Water extraction was found to reduce simmondsin and simmondsin 2' ferulate in jojoba meal to 0.08 percent (Verbiscar et al., 1980). Microbial detoxification by *Lactobacillus acidophilus* B629, with the addition of ammonia, was reported to reduce total toxicant levels to 2-5 percent (Verbiscar et al., 1980).

The major pathway of cyanide detoxification in humans and animals is via the cyanide-thiocyanate sulphur transferase (rhodanase) enzyme pathway. A less toxic thiocyanate is formed from cyanide with the addition of organic sulphur. Thiocyanate then can easily be excreted in the urine. The donated sulphur generally is obtained from sulphur containing amino acids, such as methionine, which reduce the quality of the protein available for body growth. High levels of thiocyanate can be seen in the blood and urine and one of the "side effects" of the consumption of jojoba meal in livestock is reduced weight gain and methionine deficiency (Oke, 1979). However, more recently it has been discovered that rhodanase can only protect against low doses of HCN . With the observed distribution of rhodanase in tissues and its subcellular location, the enzyme is now thought to be a part of a multienzymatic system involved in the regulation of the sulfane pool (Westley, 1980)

Determination of Cyanide

Cyanide toxicity in plants was first recognized when, over a century ago, General Kitchener's army's transport animals suffered heavily from poisoning after grazing on herbage of the Upper Nile. The Imperial Institute began researching the phenomenon and published a series of papers which isolated cyanogen compounds in *Lotus arabicus* and *Sorghum vulgare*. This led to a systematic study of cyanogenesis in plants. In Mauritius (1884) David & Stevenson give the earliest description of cyanide toxicity in man, from ingestion of *Pois d' Archery* (*Phaseolus lunatas*). This led the researcher to establish a method for determining the cyanide in plants (Oke, 1979).

Several methods have been studied to determine quantitatively the amounts of free and bound cyanides in biological and waste material (Mendoza

et al., 1984). The determination of cyanide has been studied in cassava (Mendoza et al., 1984), soybean products (Honig et al., 1983), apricot kernels (Egli, 1977), human food and animal feed (Peter van Eden & de Jong, 1985), and dried sorghum leaves (Haskins et al., 1988). The methods for estimating concentrations of cyanoglycosides depend on three stages: 1) hydrolysis of the cyanogenic glycoside; 2) isolation or trapping of released cyanide; and 3) determination or estimation of the cyanide using chemical reactivity or physical behavior of the cyanide ion (Mendoza et al., 1984).

The standard method for determining cyanoglycosides in beans can be found in the AOAC method 26.115 (1975). The cyanoglycoside in the sample is steam-hydrolyzed and the evolved HCN is collected in a solution of sodium hydroxide. With a standard silver nitrate solution, the collected cyanide is measured by titration (Kupchella & Syty, 1984). The methods given in AOAC 26.135 and 26.136 (1980) involve the decrease of titratable silver ions by a solution of potassium thiocyanate or the titration with silver ions in alkaline solution as mentioned previously. However, these methods were found to have insufficient sensitivity and overlook the quantitative liberation of cyanide from cyanogenic compounds (van Eden & de Jong, 1985).

Various instrumental techniques have been applied to the determination of cyanoglycosides including spectrophotometry, potentiometry, liquid chromatography, nuclear magnetic resonance spectrometry, chemical ionization mass spectrometry, and gas chromatography (Kupchella & Syty, 1984).

A frequently used procedure to determine cyanide in the laboratory is the Kroller method. This method has been used primarily in the determination of

residual cyanide in fumigated food and is considered to be reliable for many types of food and animal feed in addition to products containing cyanoglycosides. However, the method must be followed precisely in order to obtain reproducible results. Cyanide is converted into cyanogen bromide which reacts with pyridine to give glutaconic aldehyde. The glutaconic aldehyde reacts with benzidine to yield a purplish-red compound. The Kroller method has distinct disadvantages both colorimetrically and analytically. The colorimetric reagents used are bromine which is highly toxic and benzidine which is carcinogenic. Also, the colored reaction product has limited stability. The analytical result obtained from the liberation procedure depends strongly on the conduct of the experimenter. The modified Kroller method, the substitution of barbituric acid and N-chlorosuccinimide for benzidine and bromine, yielded approximately 50% higher sensitivity. The detection limit for cyanide in a solution was found to be approximately 10 ug/l or 10 ppm (van Eden & de Jong, 1985)

van Eden & de Jong (1985) conducted an experiment on the calibration graphs of standard solutions and analytical results of one sample of fodder beans to compare the colorimetric procedures of the Kroller method and Lambert et al. modified Konig reaction, respectively. The reproducibility of both colorimetric reactions was found to be similar, however, the barbituric acid colorimetry (Lambert et al.) was found to have a higher sensitivity than benzidine colorimetry (Kroller). The main variable influencing the reliability of analytical results during the liberation process was temperature.

First described in dye synthesis by Konig, the reaction of cyanogen halides to cleave pyridine to produce glutaconic aldehyde has been applied to

the determination of cyanide by oxidation of CN^- to a cyanogen halide, CNX , in which CN^+ is the reactive species. Cyanide is converted into cyanogen chloride by reaction with N-chlorosuccinimide. The CNCl reacts with pyridine to produce an intermediate which hydrolyzes to form the conjugated dialdehyde, glutaconic aldehyde. The glutaconic aldehyde is then coupled with a primary amine or a compound containing reactive methylene hydrogens (RH_2). N-chlorosuccinimide, stabilized with excess succinimide acts as the oxidizing solution while pyridine-stabilized barbituric acid acts as a coupling component (Lambert et al., 1975). The mechanism of the coupling reaction is as follows: barbituric acid reacts with glutaconic aldehyde to yield structures which are stabilized by resonance. Compounds such as barbituric acid capable of acquiring a positive charge or charges as a result of resonance have high molar absorptivities and produce the most sensitive methods. The stabilizing effect of excess succinimide on N-chlorosuccinimide is not completely understood as yet, but the reagent has proven effective in the oxidation of CN^- to CN^+ . Lambert et al. (1975) states that the described reagents are stable for at least six months, though, van Eeden and de Jong (1985) found that the reagent solutions are stable for one week when kept cool in a well-closed brown bottle.

Most methods for the analysis of cyanide involve the acidification of the sample followed by extended collection of the evolved HCN in a suitable trap. The trapped HCN is then analyzed using techniques including colorimetry or conversion to a compound which may be chromatographed such as cyanogen chloride (Macy, T.D., & Loh, A., 1980). NaOH is used as an appropriate trap, whereby HCN is converted to NaCN and H_2O .

Colorimetry has been used in the determination of cyanide from cyanoglycosides by many researchers for its quick and relatively simple procedure. In 1985, van Eeden and de Jong combined the liberation procedure according to Kroller with the barbituric acid colorimetry according to Lambert et al., to determine cyanide in fodder beans. The colorimetric method has been applied to water and waste water (Broderius, 1981), food components such as tapioca (cassava) (Mendoza et al., 1984)), soybean and soybean products (Honig et al., 1983), mature sorghum leaves (Haskins et al., 1988), as well as food products such as marmalade, almond paste and marzipan. Presently, studies on jojoba meal require a long, tedious process of measuring the cyanoglycoside simmondsin by HPLC. Patel et al. (1992) was the first to apply this method of cyanide determination to jojoba meal. The colorimetric method for determining cyanide in cyanoglycoside containing samples first used by Lambert et al. (1975) then modified by Haskins et al. (1988) was applied to jojoba meal. This study will attempt to determine the optimal conditions for the use of the colorimetric procedure to measure the amount of cyanide released from jojoba meal on hydrolysis with combined alkali-heat treatments.

CHAPTER 2
JOURNAL ARTICLE

Authors' Title Page
APPLICATION OF COLORIMETRIC ASSAY
FOR THE DETERMINATION OF CYANIDE IN
DEOILED JOJOBA MEAL

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ABSTRACT

The colorimetric method developed by Lambert et al. (1975) and modified by Haskins et al. (1988) for the determination of cyanide was applied in the determination of total cyanide in deoiled jojoba meal. Patel et al. (1992) preclusive investigation found this method can be utilized in jojoba meal. The procedure involves grinding the dry deoiled jojoba meal, extracting the cyanogenic glycoside simmondsin with water, combining alkali and heat of the extract and colorimetric determination of cyanide. Applicability of the procedure was tested using pure simmondsin. Since our preliminary data showed that results obtained from jojoba extracts are influenced by the number of extractions, amount of NaOH, and heating time, these parameters were optimized. Results obtained showed a concentration of 1.6 milliequivalent NaOH to be the optimal concentration of alkali in the liberation of cyanide in deoiled jojoba meal. Maximum cyanide levels were attained after a 3 minute heating in a boiling water bath. To obtain total cyanide content, at least three extractions of a single sample of jojoba meal is required. Based on a standard curve of KCN (100 ppm) solution, total cyanide content of deoiled jojoba meal determined by this method ranged from 2282 to 3250 ppm. Researchers studying the utilization of jojoba meal for a livestock ingredient can apply this simple and reliable method to the determination of cyanide in detoxified jojoba meal.

Key words: Simmondsin; jojoba; cyanide; colorimetric

INTRODUCTION

Jojoba (*Simmondsin chinensis*) is an evergreen shrub native to the Sonora desert which is now being grown in many arid and semi-arid parts of the world for its oil containing nuts. Jojoba is presently cultivated for its seeds which contain a liquid wax similar in properties to sperm whale oil. The protein-rich residue or meal remaining after oil extraction has been investigated for its utilization as a potential feed ingredient for livestock and poultry. However, jojoba meal has been shown to produce an inhibition of food intake and adverse effects on growth efficiency and total food intake in rodents and chickens (Elliger et al., 1973; Cokelaere et al., 1992; Arnouts et al., 1993). The toxicity of jojoba meal has been attributed to a closely related group of cyanogenic compounds, simmondsin and its cyanomethylenecyclohexyl glycoside analogues (Booth et al., 1974; Verbiscar et al., 1980). The mechanism of the toxicity of simmondsin is still unknown. However, the toxic effect of simmondsin is generally regarded as its ability to inhibit food intake. Manos et al. (1986) was unable to find simmondsin in the blood, kidney, liver or muscle of lambs fed rations containing 5 to 10% nondetoxified jojoba meal. The animals were leaner than the controls, but no organ damage was evident. These researchers concluded that simmondsin might be metabolized (detoxified) by bacteria in the gut of ruminants. Elliger et al. (1973) theorized that the toxicity of simmondsin might be due to the formation of the phenylacetonitrile derivative which is metabolized to cyanide. However, Cokelaere et al. (1992) found no indications that food intake reduction was caused by the liberation of HCN during the metabolism of simmondsin in rats

injected subcutaneously with 250 mg of simmondsin/kg of body weight; concentrations of CN⁻ and SCN⁻ in the blood were not elevated.

The mechanism for the liberation of cyanide in edible plants containing cyanoglycosides is well recognized; the plants are crushed to release the endogenous hydrolytic enzymes found in the cellular compartments. Colorimetric methods have been used in the determination of total cyanide in dried sorghum leaves (Haskins et al., 1988), fodder bean (van Eeden & de Jong., 1985) and cassava (Mendoza et al., 1984) for their simplicity and effectiveness. The determination of cyanide has not been used in jojoba meal because cyanide is not released by the traditional colorimetric methods cited. Simmondsin is a more complex cyanoglycoside and is resistant to hydrolysis. Cyanide can only be measured colorimetrically while in free form and the cyanide found in jojoba meal is generally tightly bound to the simmondsin molecule. While experimenting with various techniques for the cyanogenesis of jojoba meal, Patel et al. (1992) found that cyanide can be measured colorimetrically when heated with alkali. Various methods have been described to detoxify jojoba meal (Verbiscar et al., 1980, 1981), and chromatographic methods have been applied to the determination of simmondsin (Van Boven et al., 1993, 1994; Cokelaere et al., 1992). However, methods applied in the determination of cyanide from the cyanoglycosides found in jojoba meal have not been studied. For a greater understanding of the mechanism of toxicity of jojoba meal the need for a rapid and reliable method for cyanide analysis is evident.

The objective of this study was to establish the optimal conditions for the colorimetric procedure of Lambert et al. (1975) as modified by Haskins et al.

(1988) applied to the determination of cyanide in deoiled jojoba meal. The total cyanide content of deoiled jojoba meal was determined using the optimal parameters obtained for this method.

MATERIALS AND METHODS

Reagents. Succinimide, N-chlorosuccinimide, pyridine and barbituric acid used for the procedure of Lambert et al (1975) were obtained from Sigma Chemical Company. Deoiled jojoba meal samples were also obtained from Sigma Chemical Company. Pure simmondsin was obtained from Dr. Anthony J. Verbiscar, Anver Bioscience Design, Inc., Sierra Madre, CA. Substances could directly or indirectly interfere with the colorimetric measurement of HCN. Liquid soap at concentrations greater than 0.15 ml/L or granular soap at greater than 33 mg/L could displace HCN by diffusion (Broderius, 1981). All glassware was triple rinsed to avoid trace mineral & ion interaction. Pyridine-Barbituric acid solution was found to be not stable for more than 2-3 days.

Standards for Calibration. For the KCN standards, 100 ppm stock was prepared by dissolving 0.025g KCN in 100 ml water. Working standards (0.1-1.0 ppm) were made by appropriate dilutions of the stock standard.

Sample Preparation. Deoiled jojoba meal (0.5 g) was homogenized in 10 ml of distilled water using a Potter Elvehjem homogenizer driven by an electric drill for five minutes. The extract was vacuum filtered using a porcelain Buchner funnel.

Maximum Wavelength. The absorbance of samples were measured using UV 160 Shimadzu recording spectrophotometer Initially samples were

read at an absorbance of 580nm. On repeated experiments data was not reliable. The samples were scanned to determine maximum absorbance. The maximum absorbance for cyanide in jojoba meal with colorimetric reagents was observed at 582.5 nm. When measuring samples at a wavelength that is not the maximum, the observed absorbance for various samples were different with each experiment depending on where the absorbance was on the slope of the peak. Determining the maximum wavelength for cyanide in deoiled jojoba meal produced more reliability between samples.

Number of Extractions The effect of multiple extractions of jojoba meal was investigated. The residue from the first extraction was scraped from the filter paper and washed with 10 ml distilled water and the above procedure was repeated three times. The filtrate was combined with water and diluted to 50 ml in a volumetric flask, for the final experiments utilizing 1.6 milliequivalents NaOH.

Determination of Optimal Amount of NaOH on the Formation of Cyanide in Pure Simmondsin. The optimal amount of alkali on the formation of cyanide in pure simmondsin was determined: 10 mg pure simmondsin was combined with 10 ml water and 1.0 ml aqueous extract was treated with varying aliquots (0.2-1.0 ml) of 1.0 N or 2.0 N NaOH, respectively. The test tubes were capped and heated in boiling water. Test tubes were removed at various time intervals and were immediately cooled to room temperature in a cooling water bath. The samples were then analyzed for cyanide using the pyridine-barbituric acid method (Lambert et al., 1975) as modified by Haskins et al. (1988). The absorbance for color development was measured and the maximum absorbance was obtained.

Determination of Optimal Heating Time on the Formation of Cyanide in Deoiled Jojoba Meal. The optimal heating time for the liberation of cyanide in deoiled jojoba meal was determined. Typically, 0.1 ml aqueous extract of jojoba meal was combined with 0.8 ml 2 N NaOH plus 0.2 ml water and heated in boiling water. Samples were removed from boiling water bath at various heating times and colorimetrically analyzed for cyanide. The absorbance for developed color was obtained and the sample with maximum absorbance was noted. This experiment was repeated with several samples and varying amounts of jojoba meal to determine reliability and reproducibility.

Colorimetric Procedure. The following procedure reflects the results of numerous preliminary experiments in which optimal conditions for this method were determined. The applicability of the procedure was tested using pure simmondsin and applied to jojoba meal. The amount of cyanide in the aqueous extract of pure simmondsin and jojoba meal was determined colorimetrically using the pyridine-barbituric acid method (Lambert et al., 1975) as modified by Haskins et al. (1988). Aliquot (0.9 ml) of combined jojoba meal and 2 N NaOH was treated with 0.5 ml of 10 N acetic acid followed by 5 ml of solution containing 2.5 g of succinimide and 0.25 g of N-chlorosuccinimide in 1 L of water. The color was developed by adding 1 ml of solution containing 6 g barbituric acid, 30 ml pyridine and 70 ml of water. The mixture was allowed to stand for 20 minutes at room temperature and absorbance was read at 582.5 nm against 2 N NaOH plus the color reagents as a blank. Amount of cyanide liberated was determined from a standard curve prepared from a 4×10^{-3} M KCN (100-ppm) solution.

RESULTS AND DISCUSSION

Effect of Multiple Extractions on the Determination of Cyanide in Jojoba Meal. Preliminary experiments were conducted to determine how many extractions were required for total liberation of cyanide in jojoba meal. Based on earlier determinations (Patel et al., 1992), single extractions yielded low proximate values of cyanide content. It was concluded that a single extraction of jojoba meal was not sufficient to extract all of the existing cyanoglycosides from the meal. The method was altered in an attempt to determine the amount of cyanide remaining in the discarded residue. The sample was extracted four times and each filtrate was measured colorimetrically for cyanide. The summation of the absorbance of the first three extractions are shown in Figure 4. The third extract yielded an absorbance of 0.03 and the fourth extract did not show a positive color reaction for cyanide. It was determined that a minimum of three extractions yielded the best proximate value of cyanide in a sample of jojoba meal.

Effect of Heat Treatment on the Liberation of Cyanide in Pure Simmondsin and Deoiled Jojoba Meal. The Haskins et al. (1988) modified colorimetric method does not require the treatment of heat for the determination of cyanide in dried sorghum leaves. On the contrary, the application of heat to pure simmondsin and deoiled jojoba meal under alkaline conditions has been found to be integral for the colorimetric reaction to take place. Figure 5 shows the absorbance spectra of varying aliquots of jojoba meal treated with 1.6 milliequivalents NaOH. All of the test tubes, with the exception of tube (F) were heat treated for 3 minutes. Tubes A through E

displayed a maximum absorbance at a wavelength of 582.5; indicating the wavelength for cyanide. The test tube withheld from heat treatment (F) produced no colored reactant when combined with the colorimetric reagents and did not produce an absorbance peak when scanned. Based on the absorbance spectrum, it is observed that there is no free cyanide present in this sample. Heat treatment is required for the release of cyanide in pure simmondsin and jojoba meal combined with alkali.

Apparently, combined alkali and heat treatment produces a cyanide derivative from simmondsin which reacts with the cyanide color reagents. The colorimetric reagents will develop color only if the sample is able to form a cyanogen halide with the addition of chlorosuccinimide. Therefore, the cyanohydrin portion of simmondsin is hypothesized to be in free cyanide form such as HCN or CN^- . Since hydrocyanic acid is highly volatile, it is typically measured by diffusion. These conditions do not allow for the formation of HCN. Therefore, it is proposed that the cyanide measured colorimetrically is in the form of simple cyanides such as NaCN. Broderius (1981) describes methods to determine free cyanide which require acidification of the sample in dilute alkali to convert simple cyanides and metalocyanide to HCN since only free cyanide can react with the colorimetric reagents. It is assumed that the nitrile portion of simmondsin is cleaved by the heat treatment and then reacts with the Na^+ ion of the NaOH in the aqueous extract.

From the results obtained, an observed intermediate cyanide derivative is formed from simmondsin upon NaOH and heat treatment which reacts with the color reagent. The degradation of simmondsin under alkali conditions was reported to yield 2-hydroxy-3,4-methoxyphenylacetonitrile; however, in the

absence of heat (Elliger et al., 1973). Essentially, this compound is the remaining nitrile aglycone after the glucose has been hydrolyzed from simmondsin. Yet, the final product formed is unknown

Effect of NaOH Concentration on Cyanide Formation in Pure Simmondsin. In this comparison, the concentration of NaOH was varied to determine the effect on liberation of cyanide. Our preliminary tests on pure simmondsin with 1.0 N NaOH showed a positive reaction to the cyanide color reagent, yet, with low color development. By increasing the concentration of NaOH to a normality of 2.0 and altering the amount, we found a much higher development of color and, hence, a greater liberation of cyanide. Figure 6 shows the absorbance of pure simmondsin treated with varying aliquotes of 2.0 N NaOH. A heating time of 40 minutes was used. Maximum cyanide levels were obtained with 1.6 milliequivalents of NaOH with absorbance decreasing at 2.0 milliequivalents of NaOH.

Effect of Heating Time on Cyanide Formation in Pure Simmondsin. In order to determine the optimal heating time for the liberation of cyanide, a series of experiments were conducted with pure simmondsin and 1.6 milliequivalents NaOH. Figure 7 shows the effect of heating time in boiling water on the cyanide levels determined from pure simmondsin. Similar cyanide levels were attained after 40 minute and 60 minute heating time in boiling water bath. Cyanide levels of 4.65 ppm and 4.76 ppm, respectively, were detected in 1.0 ml pure simmondsin.

Total Cyanide Liberated from Pure Simmondsin. The total cyanide levels attained in pure simmondsin treated with 1.8 milliequivalents NaOH with varying time of heat treatment are shown in Table I. Liberated

cyanide levels peaked after 40 minutes boiling time and leveled at 60 minutes heating. The sample heat treated for 40 and 60 minutes showed a cyanide level of 4.65×10^{-3} and 4.76×10^{-3} mg/ml, respectively. The results of amount NaOH on the liberation of cyanide in pure simmondsin are shown in Table II. The sample treated with 1.6 milliequivalents NaOH showed a maximum of 6.76×10^{-3} mg cyanide in 1.0 mg pure simmondsin from Anver Bioscience, Inc. The analytical value for cyanide in simmondsin was calculated to be 6.9×10^{-2} mg cyanide in 1.0 mg simmondsin which produced a 9.79% yield for this sample. P

Effect of NaOH Concentration on Cyanide Formation in Deoiled Jojoba Meal. Earlier experiment on the effect of NaOH concentration on the liberation of cyanide in jojoba meal proved to be inconclusive. Figure 8 show the results of varying the NaOH amount on the development of color in jojoba meal. Because the concentration of NaOH was too dilute, (a normality of 1.0 was used for the dilutions), the data obtained was indeterminate. After a series of tests on pure simmondsin, an amount of 1.6 milliequivalents NaOH was determined to be the optimal concentration for the maximum liberation of cyanide in jojoba meal.

Effect of Heating Time on Cyanide Formation in Deoiled Jojoba Meal. A series of experiments were carried out on the effect of heating time on cyanide liberation of jojoba meal. Preliminary tests on jojoba meal extract with 0.9 milliequivalents NaOH showed maximum cyanide levels attained after a 2 minute heating time in a boiling water bath (Figure 9). Subsequent repeated tests indicated a slightly longer boiling time with maximum cyanide levels attained after 3 minutes heating time (Figure 10). Figure 11 shows the effect of heating time on the combined filtrate of 3

extractions of jojoba meal. As expected, the absorbance is higher than that of the previous test with a maximum absorbance attained at 3 minutes boiling time. The optimal heating time for jojoba meal was different than that of pure simmondsin on the formation of cyanide.

Total Cyanide Liberated from Deoiled Jojoba Meal. Data presented in Table III show the amount of cyanide liberated from 3 extractions of jojoba meal treated with 1.6 milliequivalents NaOH after 3 minutes boiling time. The calculated mean of 5 determinations for this method was 2543 ppm with a standard deviation of 360 and a range of 2282 to 3250 ppm. The cyanide levels are much higher for the method involving 3 extractions of jojoba meal as well as the determined optimal NaOH amount of 1.6 milliequivalents. As expected, the values obtained for total cyanide content with this method are higher than previous reported values (Patel et al., 1992). Patel et al. (1992) obtained cyanide values in the range of 1121 to 1548 ppm when using a method of one extraction of jojoba meal combined with 0.9 milliequivalents NaOH and 2 minutes heating time.

Relationship of Cyanide Absorbance to Varying Amount of Jojoba Meal Extract. In order to determine the reliability of this method, an experiment was performed on varying amounts of jojoba meal extract. Aliquots, (0.02, 0.04, 0.06, 0.08, and 0.1 ml) of jojoba meal extract were combined with corresponding aliquots of water, (0.98, 0.96, 0.94, 0.92, 0.90 ml) and 1.6 meq NaOH. The samples were heated in boiling water for 3 minutes and analyzed colorimetrically for cyanide. Figure 12 shows the linear relationship between amount of sample and absorbance. As expected, as the sample amount increased, the observed absorbance increased, also. The

absorbance increased, also. The results indicate a positive linear relationship between amount of sample and absorbance which demonstrates the reliability of this method.

Based on data obtained in this study the colorimetric method by Lambert et al. (1975) as modified by Haskins et al. (1988) can be applied to the determination of cyanide in jojoba meal. Extracting 0.5 g jojoba meal 3 times, treating the sample with 1.6 milliequivalents NaOH and heating for 3 minutes in a boiling water bath yields the optimal absorbance. This method can be used in the determination of total free cyanide in jojoba meal. Researchers studying the utilization of jojoba meal for a livestock ingredient can apply this method for the determination of cyanide in detoxified jojoba meal. This method can also be used to follow changes in simmondsin content during the detoxification process.

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Figure 4. Absorbance of the summation of three extractions from single sample of jojoba meal treated with 0.9 milliequivalents NaOH after heating for 3 minutes.

Figure 5. Absorbance spectra of the color of cyanide in varying aliquotes of jojoba meal treated with 1.6 milliequivalents NaOH: 0.10 ml extract (A), 0.08 ml extract (B), 0.06 ml extract (C), 0.04 ml extract (D), 0.02 ml extract (E) after 3 minute boiling time and 0.10 ml extract without heat treatment (F).

Figure 6. The effect of NaOH concentration on the liberation of cyanide in pure simmondsin at 10 minute boiling time.

Figure 7. The effect of heating time on the liberation of cyanide in pure simmondsin.

Figure 8. The effect of NaOH concentration on the development of color in deoiled jojoba meal.

Figure 9. The effect of heating time measured in second intervals on the formation of cyanide in jojoba meal treated with 0.9 milliequivalents NaOH.

Figure 10. The effect of heating time measured in minute intervals on the formation of cyanide in jojoba meal treated with 0.9 milliequivalents NaOH.

Figure 11. The effect of heating time measured in minute intervals on the formation of cyanide in 3 combined extractions of jojoba meal treated with 0.9 milliequivalents NaOH.

Figure 12. The correlation of cyanide liberated from varying amounts of jojoba meal treated with 1.6 milliequivalents NaOH and heated for 3 minutes.

Table I. Total cyanide liberated from 1.0 mg pure simmondsin heated at varying time and treated with 1.8 milliequivalents NaOH.

Heating time	Cyanide (mg/ml)
10	$2.80 \times 10^{-3} \pm 0.02$
20	$2.76 \times 10^{-3} \pm 0.04$
40	$4.65 \times 10^{-3} \pm 0.14$
60	$4.76 \times 10^{-3} \pm 0.03$

mean $\pm$ standard deviation of two determinations.

Table II. Total cyanide liberated from 1.0 mg pure simmondsin treated with varying amount of NaOH and heated for 40 minutes.

NaOH (milliequivalents)	Cyanide (mg/ml)
0.4	2.22×10^{-3}
0.8	1.83×10^{-3}
1.2	1.76×10^{-3}
1.6	6.76×10^{-3}
2.0	6.11×10^{-3}

Table III. Total cyanide liberated from 3 extractions of jojoba meal treated with 1.6 milliequivalents NaOH after 3 minutes heating time.

Trial number	Cyanide (ppm-dry basis)
1	3250
2	2302
3	2282
4	2400
5	2480

$\bar{X} = 2543$

Standard deviation = 360

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CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Researchers are interested in the effect simmondsin exerts on test animals. Currently, there exists no quick and reliable method for determining total cyanide and therefore determining simmondsin. High performance liquid chromatography, which is a lengthy and time consuming procedure, is the most common method for measuring simmondsin in the laboratory. The colorimetric method for determining total cyanide enables the researcher a quick and easy alternative to applying instrumental techniques in the determination of simmondsin.

The optimal conditions were determined for the colorimetric method of cyanide determination in deoiled jojoba meal. The results indicate that a concentration of 1.6 milliequivalents NaOH is required for the maximum cyanide liberation in pure simmondsin. The effect of heat treatment was studied. The optimal boiling time for the liberation of cyanide in pure simmondsin was found to be 40 minutes. The maximum absorbance peak at 224 nm obtained from the spectra of the sample is hypothesized to be due to the nitrile chromophore present in the cyanide compound liberated from deoiled jojoba meal samples. Elliger et al. (1973) showed the basic degradation of simmondsin to be 2-hydroxy-3-methoxyphenylacetone nitrile by way of n.m.r. spectrum. Further hydrolysis of the nitrile was shown to produce 2-hydroxy-3-methoxyphenylacetic acid and the corresponding lactone. Neither of these compounds contain cyanide. However, the color development of cyanide

obtained from pure simmondsin shows that cyanide was formed from the aqueous extract of jojoba meal plus alkali under heat treatment conditions. The second absorbance peak at 290 nm obtained from the spectra of the heated samples is postulated to be a simple cyanide such as NaCN formed from the hydrolysis of the nitrile with the addition of heat. Sodium cyanide easily reacts with the addition of acid to form HCN (Broderius, 1981). Free cyanide in the form of HCN can then be oxidized by chlorosuccinimide. The intermediate formed reacts with pyridine to produce a conjugated dialdehyde. The glutamic aldehyde is coupled with barbituric acid to produce a colored species.

Typically, low levels of cyanide (microgram per liter) are measured colorimetrically in the form of free cyanide (HCN) with methods utilizing distillation, a microdiffusion cell, or gas permeable membrane. These procedures attempt to isolate and concentrate HCN in dilute alkali. The acidification of the sample converts simple cyanides and some portions of certain metalocyanides to HCN in order to measure total cyanide. These methods are tedious to perform but are found to evaluate cyanide content successfully at low levels in the detoxified jojoba meal. The direct addition of alkali and heat treatment of jojoba meal was found to be highly effective in the determination of total cyanide content, yielding higher values of cyanide than other methods and requiring less time to perform (Patel et al., 1992).

Total cyanide content of jojoba meal was determined colorimetrically using the preliminary methods investigated by Patel et al. (1992). The procedure was then modified in an attempt to determine the optimal conditions required for total cyanide liberation. Standard cyanide solutions were prepared and analyzed spectrophotometrically to establish a calibration curve for each

set of test conditions. Maximum cyanide levels in jojoba meal were obtained after extracting jojoba 3 times, combining with 1.6 milliequivalents NaOH and heating for 3 minutes. The cyanide values ranged from 2282 ppm to 3250 ppm.

Recommendations

Since simmondsin is an unusual cyanoglycoside and cannot be compared to most other cyanoglycosides found in plants, more research is needed in the understanding of its hydrolysis and the compounds produced. The extent of hydrolysis of jojoba meal can be studied by treatment with acid and base to analyze the compounds produced (Elliger et al., 1973). Though, the authors were not specifically studying the hydrolysis of simmondsin, they analyzed degradative material in an attempt to isolate the structure of simmondsin. Further research of the colorimetric method with the addition of a buffer to prevent shifts in pH is needed. The colorimetric method referred to here is sensitive to fluxuation in pH. The liberation of cyanide from other cyanoglycosides has been found to be attributed to the release of endogenous enzymes within the cellular compartments when the plants are crushed. Whether the presence of an endogenous enzyme facilitates the hydrolysis of simmondsin is not known. Further research on the effect of an endogenous enzyme in the hydrolysis of simmondsin in jojoba meal would assist to elucidate the mechanism for toxicity, thereby providing clues to how it is metabolized in the body.

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