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Chemical composition and antinutritional factors of *Lycianthes synanthera* leaves (chomte)

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Abstract

The leaves of *Lycianthes synanthera* (chomte), an edible Guatemalan plant, were analysed for some nutritional and antinutritional factors. Results of this study indicated that chomte leaves are rich sources of Ca, K, Fe, Zn, Cu, ascorbic acid, riboflavin, crude protein, carbohydrates and energy. Chomte leaves had hemagglutinating activity, trypsin inhibiting activity of 43.2 ± 1.0 TIU mg protein^{-1} and α-amylase inhibiting activity of 3.2 ± 0.9 AIU mg protein^{-1}. A cooking treatment, 15 min boiling, resulted in a considerable decrease in antinutritional factors.

Keywords: *Lycianthes synanthera* leaves; Chomte; Proximate composition, minerals and vitamins; Antinutritional factors

1. Introduction

Food availability in Guatemala is poor, mainly due to vulnerability to environmental factors, such as droughts and floods, deforestation and soil erosion and to inadequate agricultural and economic policies. Imports have increased, mainly for cereals, milk and animal fats. The food groups that sustain the population continue to be cereals (mainly maize), sugars and beans. These foods meet nearly 90% of energy requirements and are deficient in total fats, proteins of animal origin and micronutrients, especially in low-income population groups. Therefore, it is essential that cheaper sources of protein and other nutrients be found. These could be obtained from the plant materials most in abundance, which are under-utilised. Solanaceae and Leguminosae should be given priority in this quest.

Lycianthes synanthera (Sendtn.) Bitter, commonly named "chomte" or "tiuk" in Q'eqchì, and "chilete" or "chilete dulce" in Spanish, is an edible plant of the family Solanaceae that grows naturally in Guatemala from just above sea level to 900 m (Gentry & Stanely, 1974). The leaves of *L. synanthera* are used as food by people of the Q'eqchì ethnic group who live at Alta Verapaz, Guatemala. They cut the young branches, and separate the leaves, which are boiled, drained and squeezed because of their bitter taste; then tomato, onion, salt and vegetable oil are added to make the traditional dish "chomte en chirmol". Other forms of consumption are as chomte patties, chomte boiled with black beans and "tamalitos", a traditional ball made by steamed maize dough and chomte.

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Despite the use of this edible plant among the Mesoamerican native people, there are no data in the literature concerning the chemical composition and nutritional value of *L. synanthera* leaves. Recently we isolated three new furostanol oligoglycosides, named lycianthosides A–C, together with known flavone glycosides from chomte leaves (Piccinelli et al., 2005). In the present study, the leaves were investigated to determine their proximate composition, minerals, vitamin contents and antinutritional factors. The aim of this study was to demonstrate the nutritive value and thereby to encourage an increase in the consumption and utilization of this species in Guatemala.

2. Materials and methods

2.1. Plant material

Leaves of *Lycianthes synanthera* (Sendtn.) Bitter (''chomte'') were collected near Cobàn, Alta Verapaz (200 km from Guatemala City) in July 2000 and identified by J. Castillo. A voucher sample (LS1, 2000) is deposited at the Herbario of the Facultad de Agronomia, Universidad de San Carlos de Guatemala, Guatemala.

2.2. Proximate composition analysis

The moisture content was determined by drying the leaves in a Napco 430 oven at 105 °C until a constant weight was obtained (AOAC, 1990). Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method ($6.25 \times N$) (AOAC, 1990) in a Tecator 2020 digestor and Kjeltec 1030 autoanalyzer. Fat was determined by the method described by the AOAC (1990), using the Soxhlet system (AOAC, 1990). Ash content was determined by dry ashing in a Lindberg 51442 muffle furnace at 525 °C for 24 h. Crude fibre was determined in a Tecator Fibertec 1010 fiber digester (AOAC, 1990). Carbohydrates were calculated as ''Nitrogen free extract'' according to the formula: Carbohydrates = 100 − (% moisture + % protein + % crude fibre + % fat + % ash). Energy (kcal) was calculated according to the formula: Energy = (g protein × 4) + (g carbohydrates × 4) + (g fat × 9).

2.3. Mineral analysis

For mineral determination, the samples were digested in $HNO_3/HClO_4$. The elements, Na, K, Ca, Mg, Fe, Zn, Mn and Cu, were measured by atomic absorption spectrophotometry, using a Perkin–Elmer Lambda 34044 atomic absorption spectrophotometer (Perkin–Elmer, Norwalk, CT). Phosphorus (P) was measured by the vanadate colorimetric method (AOAC, 1990). The results were expressed as absorbance at 430 nm. Standard curves were used for the determination of the elements in question.

2.4. α- and β-Carotene analysis

α- and β-Carotenes were extracted with acetone and ether, separated by open column as previously reported (Carvalho, Collins, & Rodríguez–Amaya, 1992) and assayed by HPLC on a Agilent 1100 series system consisting of a G-1312 binary pump, a G-1328A Rheodyne injector (20 µl loop), a G-1322A degasser and a G-1315A photodiode array detector, equipped with an analytical Restek LC-18 ODS amine column (24 cm × 4.6 mm, 5 µm) plus its guard column. α- and β-Carotene peaks were monitored at their spectral maxima: all-*trans*-β-carotene (452 nm); all-*trans*-α-carotene (445 nm). Mobile phase: A = 20 µm $NaClO_4$ in MeOH/H_2O (96/4, v/v), B = MeOH/2-propanol (55/45, v/v); gradient program: % B 5 for 5 min, % B 20 in 15 min and then % B 90 in 25 min; flow: 1 ml/min. Quantification was carried out by external standards. Purity of the standards was checked before use.

2.5. Ascorbic and dehydroascorbic acid analysis

Dried leaves (500 mg) were homogenized under a flow of nitrogen for 3 min with a Teflon homogenizer at the maximum speed in the presence of 3.0 ml of 0.01 M saline sodium phosphate buffer (PBS), containing 1 mM EDTA, pH 7.0. Homogenates were centrifuged at 100,000g for 30 min, at 4 °C. The supernatant fraction (assay solution) was collected for vitamin C determination. Assay solution (500 µl) were treated with 2 µg of hypoxanthine (reference standard) and two volumes of 2% metaphosphoric acid for vit C analysis and with two volumes of 2% metaphosphoric acid supplemented with 6 mg/ml dithiothreitol for total vit C (ascorbic + dehydroascorbic acids) analysis. Both samples were stored at −80 °C under argon, and centrifuged before HPLC analysis. The supernatant was collected and volume adjusted to 1 ml with water. To determine total vitamin C content, the supernatant containing dithiothreitol was incubated at 45 °C for 2 h prior to HPLC analysis. Samples of 50 µl were injected on an analytical Supelcosil LC-18-DB column (24 cm × 4.6 mm, 5 µm, Supelco) plus its guard column, by using, in line, both photodiode array detectors set at 265 nm and an ESA CoulArray (oxidation potential: +400 mV). The mobile phase consisted of 0.0 2M NaH_2PO_4/CH_3CN, 99.5/0.5, v/v, containing 0.6 g/l of metaphosphoric acid; flow was 0.6 ml/min. Ascorbic acid was quantified by comparison of areas to those of authentic standards, including the reference standard.

2.6. Thiamin and riboflavin analysis

Thiamine was extracted from the sample with dilute sulphuric acid in an autoclave and oxidized with cyanogen bromide to thiochrome, which is extracted with isobutanol. The fluorescence of thiochrome was determined by HPLC on a 5 μm HyPurity Aquastar (150 × 4.6 mm) column (C18) with fluorimetric detection, as described by Vuilleumier, Probst, and Brubacher (1988). Riboflavin was extracted from the sample in an autoclave with dilute sulphuric acid. The extract was diluted in methanol and any precipitate was removed by centrifugation. The riboflavin content was determined by HPLC on a 5 μm HyPurity Aquastar (150 × 4.6 mm) column (C18) with fluorimetric detection, according to Schuep and Steiner (1988).

2.7. Preparation of the crude extracts

Twenty five grammes of the dried plant were homogenized with 500 ml 0.15 M NaCl, filtered and centrifuged at 5000*g* for 15 min at 4 °C and the supernatant lyophilized. The protein concentration of the extracts was determined according to Bradford (1976).

2.8. Hemagglutination assay

Goat, horse, and human [A,B,O, (Rho (D)+)] erythrocytes were washed three times with 0.9% NaCl after each centrifugation at 3000*g*for 5 min and resuspended at a 5% (v/v) concentration in the same solution. The hemagglutinating activity was assayed in 96-well microtitre plates (U-shaped), 50 μl of erythrocytes suspension were added to 100 μl of the extract solution (1 mg protein/ml). The negative control was realized by adding 100 μl of NaCl 0.9% to 50 μl of erythrocytes suspension. All samples were assayed in duplicate. The agglutination was screened visually after 1 h of incubation at room temperature.

2.9. α-Amylase inhibitor activity

α-Amylase inhibitor activity was measured at pH 7.2 (saline sodium phosphate buffer, PBS) usual amyloclastic method with slight modifications: 100 l of the extract were added to 15 μl of the pancreatin solution (100 μg protein/ml in PBS). To each tube was then added 500 μl of the starch solution (500 mg/l), previously warmed to 37 °C and, exactly 5 min later, the reaction was terminated by adding 50 μl of iodine solution (5.4 mM in HCl) to each of the experimental tubes. Before the absorbance of each solution was measured at 590 nm against water, 1.5 ml of water were added to each tube. A tube with no extract and 100% amylase activity was the control tube and another one with no pancreatin solution, was the blank tube. The amylase activity was calculated by the following formula: [(Abs blank − Abs test tube)/Abs blank] × 100. One unit of α-amylase activity inhibited (AIU) was defined as the number of units of amylase inhibited per milligramme of protein in the extract.

2.10. Trypsin inhibitor activity determination

The trypsin inhibitor activity was measured by the method of Kakade, Simons, and Liener (1969) using benzoyl DL arginine-*p*-nitroanilide (BAPA) as substrate. Briefly, 0.25 ml of the extract was pipetted into two of the three test tubes; 0.25 ml of the trypsin solution (0.005% in HCl 1 mM) was added to each of the tubes. The blank tubes had 0.25 ml of 30% acetic acid (control). The test tubes were placed in a water bath at 37 °C. To each tube was then added 1.75 ml of BAPA solution (0.03% in 0.05 M Tris, 0.02 M $CaCl_2$, pH 8.2), and exactly 10 min later the reaction was stopped by adding 0.25 ml of 30% acetic acid to each of the reaction tubes. The absorbance was measured at 410 nm against water. One trypsin unit (TU) is defined as an increase of 0.01 absorbance units at 410 nm in the reaction mixture under the conditions defined herein. Trypsin inhibitor activity is the number of TU inhibited (TIU) per milligramme of protein in the extract.

2.11. Cooking treatment

To examine the cooking effect on antinutritional factors, 8 g of raw *L. synanthera* leaves were cooked for 15 min in boiled deionized water; the cooking water was removed and the plant homogenized with 150 ml of 0.15 M NaCl, then filtered and centrifuged at 5000*g* for 15 min at 4 °C and the supernatant lyophilized.

2.12. Statistical analysis

Four independent analyses were done for all determinations. Data were treated by analysis of variance. Significance was established at $P < 0.05$.

3. Results and discussion

3.1. Chemical composition

The proximate composition of *Lycianthes synanthera* leaves is given in Table 1. The crude protein content (6.3 ± 0.7%) is high when compared with common leaf green vegetables, such as lettuce, spinach, beet, cress and endive where the crude protein content is about 1.3%, 3.2%, 1.6%, 2.6% and 1.7%, respectively (Table 1) (Lenter, 1983) and make chomte leaves a good supplement for cereal-based diets. Also, the carbohydrate content (6.4 ± 0.8%) and the total soluble and insoluble

Table 1
Proximate composition of chomte and other edible raw leaves[a]

	Licianthes synanthera (chomte)	*Lactuca sativa* (lettuce)	*Spinacia oleracea* (spinach)	*Beta vulgaris* var. *cicla* (beet)	*Lepidium sativum* (cress)	*Cichorium endivia* (endive)
Energy (kcal)	54 ± 2[b]	14	26	27	32	20
Moisture (g)	82.5 ± 2.4[b]	95.1	90.7	90.8	89.4	93.1
Protein (g)	6.3 ± 0.7[b]	1.3	3.2	1.6	2.6	1.7
Fat (g)	0.4 ± 0.1[b]	0.2	0.3	0.4	0.7	0.1
Carbohydrates (g)	6.3 ± 1.2[b]	2.5	4.3	5.6	5.5	4.1
Crude fiber (g)	2.8 ± 0.6[b]	0.5	0.6	1.0	1.1	0.9
Ash (g)	1.7 ± 0.5[b]	0.4	0.7	0.6	0.7	0.1

[a] The composition of leaves refers to 100 g of raw leaves; the composition of other leaves are from Documenta Geigy (1983).
[b] Means of four determinations ± SD.

dietary fibre fractions (2.8 ± 0.6%) of the leaves are high when compared with commonly consumed leafy vegetables (Table 1). The crude fat content (0.4 ± 0.1) in the leaves of chomte is close to that in most green leaf vegetables, which is about 0.1–0.7% (Lenter, 1983). The ash content of chomte (1.7%) is significant in that it contains nutritionally important mineral elements.

The mineral analysis (Table 2) indicates a high concentration of potassium (417 mg/100 g), followed by calcium (252 mg/100 g), magnesium (75.2 mg/100 g), phosphorus (47.3 mg/100 g) and sodium (6.1 mg/100 g). These values, except for the sodium, were high compared with those of common vegetables (Table 2). The high level of Ca is very interesting. Ca intakes (mg/day) are reputed to vary widely between different regions of the world (USA and Canada 1031; Europe 896; former USSR 751; Africa 368; Latin America 476; Near East 484; Far East 305) (FAO, 1991). The lowest intakes are reported from communities, where animal milks are scarce or not habitually consumed; in these cases, contributions of non-milk foods to calcium intake could be valuable. This is particularly striking for women living in rural areas of Guatemala who have elevated requirements for many years due to repeated pregnancies and long lactational periods. The data on microelement composition indicated that the leaves of this underexploited plant are rich sources of Fe, Mn, Zn and Cu (Table 2). The Fe concentration (1.9 mg/100 g) was high when compared to those reported for beet and cress and only slightly low when compared to spinach (3.1 mg/100 g) and beet (2.7 mg/100 g), widely considered vegetables with high contents of Fe. Also, the concentrations of Mn (1.1 mg/100 g), Zn (0.54 mg/100 g) and Cu (0.38 mg/100 g) in chomte are high when compared with other leafy vegetables (Table 2), and regular consumption of chomte may help in preventing adverse effects of dietary deficiencies of these microelements.

Table 2 also shows the vitamin contents of the chomte leaves. Vitamin C was found to be 22.3 mg/100 g, which is higher than that reported for lettuce and endive, comparable with that reported for beet and lower than that reported for spinach and cress. This

Table 2
Mineral and vitamin levels of raw chomte leaves and other edible leaves[a] (mg/100 g leaves)

	Licianthes synanthera (chomte)	*Lactuca sativa* (lettuce)	*Spinacia oleracea* (spinach)	*Beta vulgaris* var. *cicla* (beet)	*Lepidium sativum* (cress)	*Cichorium endivia* (endive)
Minerals						
Calcium	252 ± 8.0[b]	35	106	110	81	44
Phosphorus	47.3 ± 0.9[b]	26	51	29	76	67
Iron	1.9 ± 0.05[b]	2	3.1	2.7	1.3	1.7
Magnesium	75.2 ± 2.5[b]	11	62	65	27	10
Manganese	1.51 ± 0.07[b]	–	–	–	–	–
Sodium	6.1 ± 0.02[b]	12	62	147	14	10
Potassium	417 ± 10.6[b]	140	662	550	606	380
Copper	0.38 ± 0.02[b]	0.07	0.20	0.11	0.12	0.09
Zinc	0.54 ± 0.02[b]	0.16	0.22	–	–	0.34
Vitamins						
Carotenes	0.311 ± 0.01[b]	0.582	4.86	3.90	5.58	1.98
Ascorbic acid	22.3 ± 0.9[b]	8	51	34	69	10
Thiamine	ND	0.06	0.10	0.03	0.08	0.07
Riboflavin	0.56 ± 0.02[b]	0.07	0.20	0.09	0.26	0.14

[a] The composition of other leaves are from Documenta Geigy (1983).
[b] Means of four determinations ± SD.

means that 250–270 g/day of the above vegetable are sufficient to meet the daily nutritional needs of healthy persons (Recommended Dietary Allowances) for vitamin C, which are 60 mg/day (National Research Council, 1989). Also, riboflavin concentration (0.56 mg/100 g) was very high compared with other leafy vegetables (Table 2). Riboflavin deficiency may be quite common in developing countries where intake of animal products is low, and especially during seasons when there is less intake of vegetables. It has been estimated that 50% of adults in Guatemala are riboflavin deficient (Boisvert et al., 1993). Our results show that regular consumption of chomte may help in preventing adverse effects of riboflavin deficiency. In developing countries, more than 80% of the dietary vitamin A is supplied by carotenoids present in plant foods. The leaves of chomte have only small amounts of α- and β-carotene (311 μg/100 g) when compared with other green leafy vegetables (Lenter, 1983) and compared with some common and less familiar foods of plant origin (Bhaskarachary, Sankar Rao, Deosthale, & Vinodini, 1995). Thiamine (B_1) was undetectable by our analytical method, indicating that, in chomte leaves, the level of this vitamin is much lower than that reported in other leafy vegetables.

3.2. Antinutritional factors

Enzyme inhibitors (trypsin, chymotrypsin and α-amylase) and hemagglutinins (lectins) are factors that exert a negative impact on the nutritional quality of the food. Lectins are a class of proteins or glycoproteins characterized by their ability to bind particular sugar residues that belong to polysaccharide moieties of glycoproteins, glycolipids, polysaccharides or simple glycosides. Lectins combine with the cells that line the intestinal mucosa and cause a non-specific interference with the absorption of available nutrients, and also reduce feed intake (Murray, 1984).

In our experiments, the raw *chomte* leaves agglutinated the erythrocytes of the three human and two animals blood groupings (Table 3), however, there was no hemagglutination when the leaves were subjected to 15 min of boiling. Protease inhibitors exert their antinutritional effect by causing a poor dietary protein utilization, which ultimately results in inhibition of the growth. The raw chomte leaves had a moderate concentration of trypsin inhibitors (43.2 TIU mg^{-1} of protein). Although the trypsin inhibitor activity (TIA) was established to be heat-labile (Khokhar & Chauhan, 1986) and cooking and autoclaving have been reported to be effective in inactivating protease inhibitors in several foods of plant origin (Deka & Sarkar, 1990; Gupta & Wagle, 1980; Monorama & Sarojini, 1982; Ologhobo & Fetuga, 1983), in the present investigation TIA was not found to be completely destroyed under heat treatments (15 min boiled), and a non-significant reduction (19%) of this activity was observed. This heat stability is probably due to numerous disulfide bonds between pairs of cysteine residues presents in the molecule (Ryan, 1981). It is possible that, for complete elimination of TIA in chomte, more severe cooking is necessary. The levels of α-amylase inhibitor activity in raw chomte is 3.2 AIU/mg protein and this activity seems to be significantly lower (−47%) after cooking treatment.

Table 3
Antinutritional factors in raw and 15 min boiled *Lycianthes synanthera* extract

Antinutritional factor	Raw chomte	Boiled material
Hemagglutinating activity		
A	H	–
B	H	–
O	H	–
Goat	H	–
Horse	H	–
Trypsin inhibitor activity (TUI/mg protein)	43.2 ± 1.0	35.1 ± 0.8
α-Amylase inhibitor activity (AUI/mg protein)	3.2 ± 0.9	1.7 ± 0.2

Means of four determinations ± SD.

4. Conclusion

Results of this study indicated that chomte leaves are rich sources of Ca, K, Fe, Zn, Cu, ascorbic acid, riboflavin, crude protein, carbohydrates and energy. This vegetable, therefore, has high potential for reducing nutrient and energy deficiencies existing in rural communities of Guatemala, if adequate amounts are consumed on a regular basis. Because of the high contents of calcium, iron and riboflavin found, many women would potentially benefit from eating chomte leaves during pregnancy and lactation. The nutritional value of the leaves are reduced, due to the presence of antinutritional factors, such as hemagglutinins (lectins) and trypsin and α-amylase inhibitors, which limit optimal utilization of some of the nutrients by the body. Traditional 15 min boiling treatments resulted in a significant reduction in lectins and α-amylase inhibitor activity in chomte, whereas trypsin inhibitor activity was not found to be completely destroyed with this heat treatment. To ensure the nutritional quality of processed chomte leaves, further investigation related to the heating times and cooking methods are in progress.

References

AOAC. (1990). *Official methods of analyses of association of analytical chemist* (15th ed.). Washington, DC: Association of Analytical Chemists.

Bhaskarachary, K., Sankar Rao, D. S., Deosthale, Y. G., & Vinodini, R. (1995). Carotene content of some common and less familiar foods of plant origin. *Food Chemistry, 54*, 189–193.

Boisvert, W. A., Castaneda, C., Mendoza, I., Langeloh, G., Solomons, N. W., Gershoff, S. N., et al. (1993). Prevalence of riboflavin deficiency among Guatemalan elderly people and its relationship to milk intake. *American Journal of Clinical Nutrition, 58*, 85–90.

Bradford, M. (1976). A rapid method for quantification of microgram quantities of protein utilization of the principle of protein dye binding. *Analytical Biochemistry, 72*, 248–254.

Carvalho, P. N. R., Collins, C. H., & Rodríguez–Amaya, D. B. (1992). Comparison of provitamin A determination by normal phase gravity-flow column chromatography and reversed-phase high performance liquid chromatography. *Chromatographia, 33*, 133–137.

Deka, R., & Sarkar, C. R. (1990). Nutrient composition and anti-nutritional factors of *Dolichos lablab* (L) seeds. *Food Chemistry, 38*, 239.

Food and Agriculture Organization of the United Nations. (1991). *Production yearbook* (Vol. 44). Rome: FAO.

Gentry, J. L., & Stanely, P. C. (1974). Flora of Guatemala. In: *Field museum of natural history* (Vol. 24, part X. p. 65). Chicago, USA.

Gupta, K., & Wagle, D. S. (1980). Changes in antinutritional factors during germination in *P. mungoreus*, a cross between *P. Mungo* (M_1) and *P. aureus* (T_1). *Journal of Food Science, 45*, 394–397.

Kakade, M. L., Simons, N., & Liener, I. E. (1969). An evaluation of natural and synthetic substrates for measuring the antitryptic activity of soybean samples. *Cereal Chemistry, 46*, 518–526.

Khokhar, S., & Chauhan, B. M. (1986). Antinutritional factors in mothbean (*Vigna acenitifolia*): varietal difference and effects of methods of domestic processing and cooking. *Journal of Food Science, 51*, 591–594.

Lenter, C. (1983). *Documenta Geigy scientific tables* (8th ed.). Ardsley, NY: Ciba-Geigy Pharmaceuticals Limited (pp. 245–247).

Monorama, R., & Sarojini, G. (1982). Effect of different heat treatments on the trypsin inhibitor activity of soybean. *Indian Journal of Nutrition and Dietetics, 19*, 8.

Murray, D. R. (1984). Accumulation of seed reserves of nitrogen. In D. R. Murray (Ed.). *Seed physiology, development* (Vol. 1, pp. 83–137). New York, USA: Academic Press.

National Research Council, (1989). *Recommended daily allowances* (10th ed., p. 284). National Academy of Sciences: Washington, DC.

Ologhobo, A. D., & Fetuga, B. L. (1983). Trypsin inhibitor activity in some lima bean (*P. lunatus*) varieties as affected by different processing methods. *Nutrition Reports International, 27*, 41.

Piccinelli, A. L., Salazar, J., Velasquez, R., Quesada, S., Aquino, R., & Rastrelli, L. (2005). Three new furostanol saponins from the leaves of lycianthes synanthera (“chomte”), an edible mesoamerican plant. *Journal of Agricultural and Food Chemistry, 53*, 289–294.

Ryan, C. A. (1981). Proteinase inhibitors. In *The biochemistry of plants* (pp. 351–367). New York, USA: Academic Press.

Schuep, W., & Steiner, K. (1988). Determination of vitamin B2 in complete feeds and premixes with HPLC. In H. E. Keller (Ed.), *Analytical methods for vitamins and carotenoids in feed* (pp. 99). Switzerland: Roche Vitamins and Fine Chemical Division.

Vuilleumier, J. P., Probst, H. P., & Brubacher, G. (1988). Determination of vitamin B1 in complete feeds and premixes. In H. E. Keller (Ed.), *Analytical methods for vitamins and carotenoids in feed* (pp. 9). Switzerland: Roche Vitamins and Fine Chemical Division.