CHAPTER II

DETERMINATION OF THE QUALITY OF TURMERIC IN THE LOCAL MARKETS

1. Introduction

Turmeric is classified as a crude drug in the Traditional Medicine System, and therefore the specifications and standard of quality are not yet established. It is then necessary to determine the average quality of turmeric available in the markets, the value of which will be used as a temporary standard for quality comparison with the experimental turmeric produced.

For the purpose, samples of Curcuma longa Linn were collected from 15 provinces of Thailand, representing specimens from every geological part.

The samples were analysed by the standard methods for the control of vegetable drugs, namely: loss on drying, volatile oil content, total ash and acid-insoluble ash, volatile and non-volatile ether extracts.

The curcuminoid pigment content was determined by the spectrophotometric method recommended by the American Spice Trade Association and the FAO Food and Nutrition Section.

The details of the methods of analyses employed were as the the followings:

1.1 Loss on drying[13,16,27]

The gravimetric method was employed. One to two grams of the powdered sample was accurately weighed in a flat weighing bottle and dried in an oven at 100-105°C for a specified time. The loss in weight of the drug represents the moisture content in the sample. Therefore, it shows the limits of water for vegetable drugs, because of the presence of excessive water in vegetable drugs will promote the growth of microbes, fungi and insects and the hydrolysis of constituents leading to deterioration of the drug. Therefore, the limits of water should be prescribed for vegetable drugs. The pharmacopoeial limits of water for vegetable drugs are usually 8-14% with few exceptions.

1.2 Volatile oil content[13,16,27]

Volatile oils are the odorous principles found in various plants and characterized by their oil-like appearance and ability to volatilized at room temperature. They are usually mixtures of terpenes, sesquiterpenes and their oxygenated derivatives. Aromatic compounds may also occur and predominate in certain volatile oil. The high percent V/W of volatile oil content is useful because of volatile oil is used in medicine, and food industries. The determination of a volatile oil in a drug is made by distilling the drug with water, collecting the distillate in a graduate tube in which the aqueous portion of the distillate is automatically separated and measuring the volume of the oil in the receiving tube.

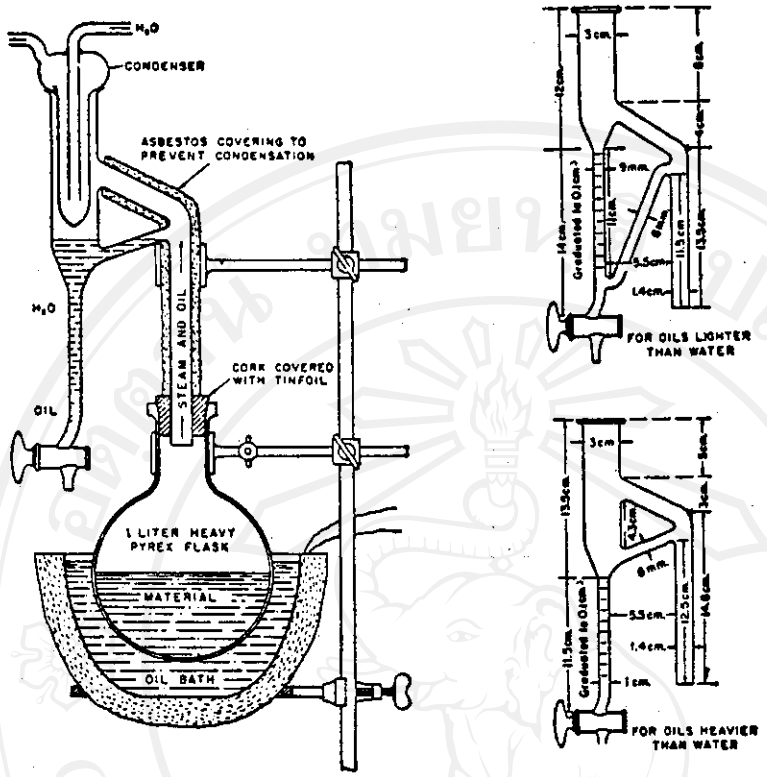


Figure 3: Volatile-oil distillation apparatus.

1.3 Total ash and acid-insoluble ash [13,16,27]

The determination of ash is a method to measure the amount of the residual substance not volatilized when the drug sample is ignited. Ash may be derived from the plant tissue itself which is usually called "physiological ash" and ash may also come from the extraneous matter, expecially sand and soil, adhering to the surface of the drug which is called "non-physiological ash". These two kinds of ash are determined together, therefore it is called

"total ash"。 Total ash is the residue obtained by the incinerating the material by gradually increasing the heat, not exceeding 450°C until free from carbon, cool in a desiccator and weigh。 The acid-insoluble ash is the residue obtained by boiling the total ash with diluted hydrochloric acid, collecting the insoluble matter in a filter, washing and igniting。 This is the method intended to measure the amount of silica especially sand and siliceous earth, present in the drug。

1。4 Volatile and non-volatile ether extracts[13,16,27]

The determination of total ether extract is a method designed to measure the drugs which contain volatile oils and the amount of a certain constituent or group of related constituents。 It is applied to those vegetable drugs for which there is no suitable method of chemical or biological assay for their active constituents at present available, while the determination of non-volatile ether extract is applied to drugs having active constituents after volatiled matters in total ether extract are volatilized and omits the extract。 But the amount of extract in a drug is only an approximate measurement。 The soxhlet apparatus is generally used for extraction with small quantities of a drug and volatile solvents。 The solvent is heated over a heating mantle because it is highly inflammable。 The weighed sample of drug is placed in a porous paper extraction thimble which is then fitted in to the extraction tube。 After the extraction is complete, the solvent is driven off until

the flask and its contents are constant weight and the percentage of extractive in the drug is calculated from the weight of the residue.

1.5 Determination of the total pigment content[9,20,21,27]

Curcumin, the principle pigment of turmeric, and other two minor related compounds absorb light in the visible region at 425 nm. For the determination of total pigment content, therefore, the spectrophotometric method is recommended by the American Spice Trade Association and the FAO Food and Nutrition Section. The method is rapid and accurate. However, the method does not differentiate the content of each compound since the absorption maxima are at the same wavelength.

The priciple of visible and ultraviolet spectroscopy is as follows:

Visible and ultraviolet spectroscopy are widely used techniques for the quanlitative and quantitative analysis of organic and inorganic systems. Many compounds or their derivatives have spectra in visible and ultraviolet region and therefore they are able to analysis by this spectroscopic techniques. The widespread use of these methods is due to many factors such as the availability of simple to use instruments at relatively low cost. These methods are generally sensitive and selective and the results are easy to interprete. Spectroscopic techniques are based on the measurement of absorption or emission of electromagnetic radiation. The absorption

on emission process is a result of an electronic rearrangement in atoms or molecule. In the absorption process the atom or molecule is first in a ground electronic state, on absorbing the incident radiation, the system rises to a higher energy electronic excited state. The emission is the reverse of the absorption process, that is a molecule already in an excited state reverts to the ground state by emission of energy in the form of electromagnetic radiation. Electronic spectra in the visible and UV regions are usually measured with a spectrophotometer. The basic elements are illumination, dispersion, and detection. In single-beam operation, the sample is examed to determine the amount of radiation absorbed at a given wavelength and the results are then compared with a standard or reference obtained in a separate examination. In double-beam operation the monochromatic beam (after dispersion) is split into two components of equal intensity. One beam passes through the sample, the other through the reference, and the difference between the two components is determined simultaneously. The most sensitive detectors used in spectrophotometers are quartz windowed photomultipliers. The response of the detector should be linear with the number of photons absorbed. The currents from the photomultipliers are amplified before being recorded. The optical diagram of the spectrophotometer is shown as Figure. 4 , page 36.

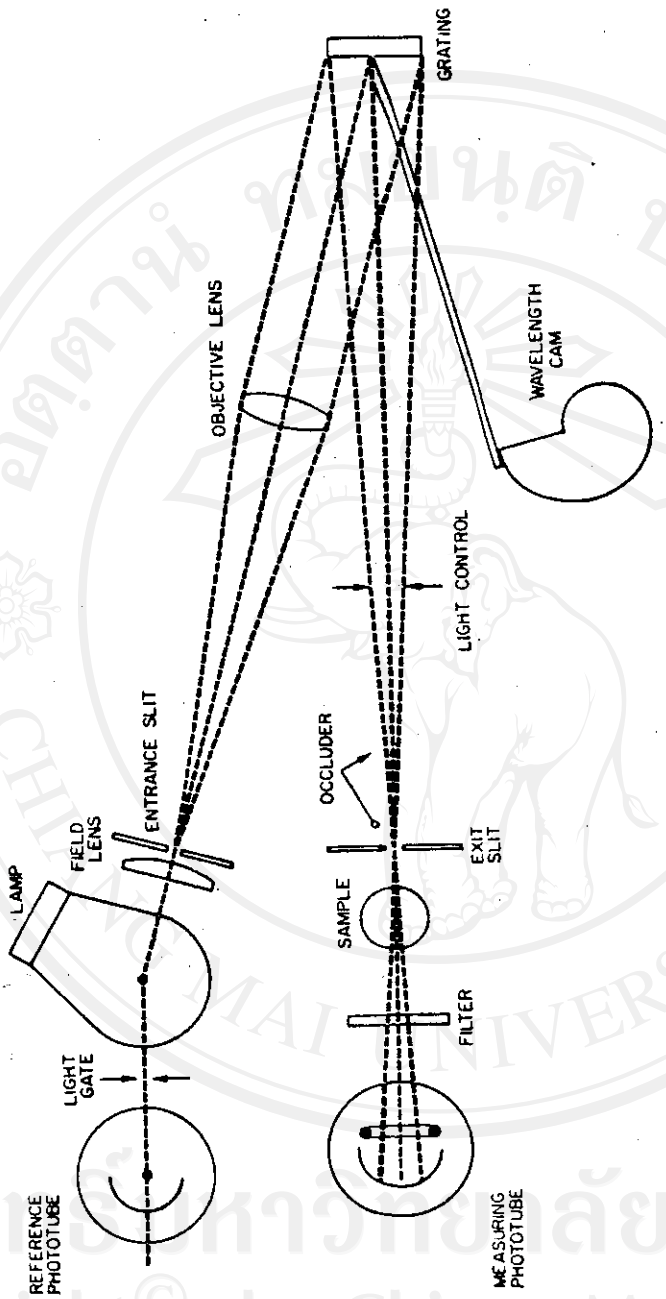


Figure 4: Schematic Diagram of the Spectronic 20. Courtesy of Bausch & Lomb, Inc., Rochester, N.Y.

2. Experimental part

2.1 Apparatus and chemicals

2.1.1 Apparatus

Aluminium pot with covered lid diameter 18 inches

Aluminium tray width x length 15 x 20 inches

Gravity convection oven from Precision Scientific group.

Muffle furnace, Thermolyne Corporation, Subsidiary of Sybron-Corporation, Dubuque, Iowa, U.S.A.

Mettler P 163, Mettler LP 12

Mill, Arthur H.Thomas Co., Phila;Pa;U.S.A.

Rotavapor-R,Büchi,Brinkmann.

Soxhlet extraction apparatus 45/50, Corning glass works, corning New York.

The Bausch & Lamb Spectronic® 20 Spectrophotometer.

Whatman Cellulose Extraction Thimbles internal diameter x external length 33 x 118 nm.

2.1.2 Chemicals

Diethylether, Riedel-Dehaenag, Seelze-Hannover.

Ethanol absolute, R.P.NORMAPUR®

Hydrochloric acid, Pronalys May & Baker Ltd.

Curcumin Fluka AG,Buchs SG.

2.2 Samples

2.2.1 Collection of samples. Turmeric was bought in the fresh form and the dried form from the market and the traditional drug store. In case of the fresh turmeric, curing of turmeric must be done.

Turmeric was collected from 15 provinces in Thailand as following

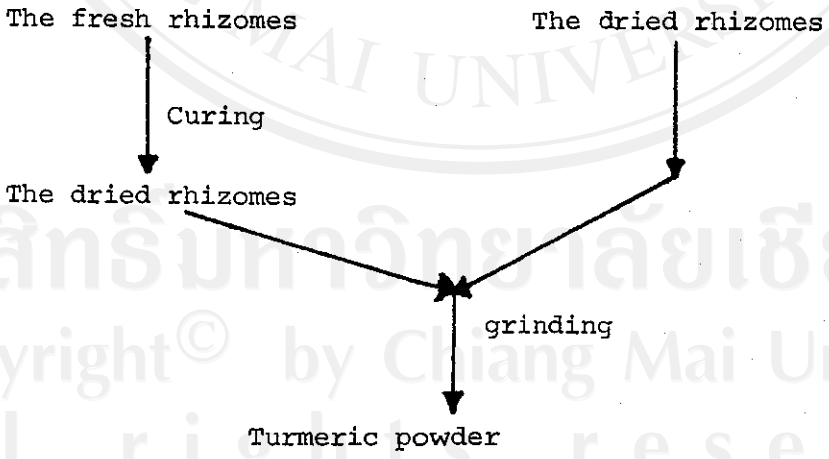
1. Amphur Hang Dong; ChiangMai, the fresh form.
2. Amphur Muang; Yala, the dried form.
3. Amphur Ban Pong; Ratchaburi, the fresh form.
4. Amphur Muang; Lamphun, the fresh form.
5. Amphur Mae Sai; Chiang-Rai, the dried form.
6. Amphur U-Thong; Suphan Buri, the dried form.
7. Amphur Muang; Phayao, the fresh form.
8. Amphur Muang; Nakhon Pathom, the fresh form.
9. Amphur Tha Mai; Chanthaburi, the fresh form.
10. Amphur Muang;Chon-Buri, the dried form.
11. Amphur Muang; Nakhon Ratchasima, the fresh form
12. Amphur Muang; Phichit, the fresh form.
13. Amphur Muang; Rayong, the fresh form.
14. Amphur Muang; Songkha, the dried form.
15. Amphur Manorom, Chai Nat, the fresh form.

2.2.2 Curing. The fresh mother rhizomes and fingers are cured separately, because the former take longer time for cooking

than the latter. The curing process is as follows: The rhizomes are put into an aluminium pot. The water is added until it covers the turmeric to a depth of 2-3 inches. The pot and contents are heated gently with lid close, until the turmeric becomes soft that takes about 1-3 hrs. The cooked turmerics in the pot are allowed to cool gradually at room temperature. Bring them out of the pot, spread as a thin layer on an aluminium tray, and let them dried in the air over night. After that the cooked turmerics are dried in an oven at temperature not exceeding 60 C^{o} until completely dried, which takes about 48 hrs.

2.2.3 Grinding. Grind the dried turmeric fingers in a mill in order to receive fine yellowish powder which passes through a 1 mm. screen. Mix turmeric powder throughly since most spices lack uniformity and tend to stratify and store in a glass-tight container.

The diagram showing the processing of curcuma collected from 15 provinces in Thailand is as follows.



2.3 Analyses (the results are shown in Table 8, page 43)

2.3.1 Determination of loss on drying. Two grams of turmeric powder is accurately weighed in a flat tray of a Mettler-P 163. Smear turmeric powder as a thin layer. Dry in a Mettler- P 163 until constant weight. The loss in weight of the drug represents the moisture content in the turmeric powder.

2.3.2 Determination of volatile oil content. Place twenty grams of turmeric powder, accurately weigh in a 2-litres round bottom flask, and fill it one-half with water. Add glass beads to the mixture to prevent bumping. Connect the flask with a reflux condenser and a receiver. Boil the contents of the flask slowly for 15 hours on a heating mantle, until distillation is complete. Leave the distillate over night and read the volume. The content of volatile oil is expressed as a percentage V/W.

2.3.3 Determination of ash content

2.3.3.1 Total ash. Accurately weigh two grams of the sample into a tared crucible. Then ash in a muffle furnace for 6 hrs at 600 C^{o}, break up the ash with several drops of water. Evaporate carefully to dryness and heat in a muffle furnace for 2 hrs until free from carbon. Then keep it in a desiccator and determine the weight of the ash in gram per 100 grams of air-dried sample.

2.3.3.2 Acid-insoluble ash. Boil the total ash (which obtained from 2.3.3.1) with 25 ml of diluted hydrochloric

acid for 5 minutes, cover the dish with watch glass to prevent spattering, then collect the insoluble matter on an ashless filter paper, wash with hot water until the washing are acid-free. Ignite 6 hrs at 600 C^{o} until carbon-free, Then keep it in a desiccator and determine the weight of the acid-insoluble ash in gram per 100 grams of air-dried sample.

2.3.4 Determination of ether extractives

2.3.4.1 Total ether extractive. Weigh ten grams of the turmeric powder into a soxhlet extraction apparatus. Extract with diethylether for 20 hrs. Transfer the ethereal solution to a tared porcelain dish and allow it to evaporate spontaneously. Then dry it over fresh, efficient desiccant in a desiccator for 18 hrs and weigh the total ether extract. Calculate the content of total ether extract in gram per 100 grams of the air-dried sample.

2.3.4.2 Volatile and non-volatile ether extractives. Heat the total ether extract gradually up to 110 C^{o}, until the weight becomes constant; the loss in weight during the heating represents the volatile portion of the extract. Calculate the content of extractive in gram per 100 grams of the air-dried sample.

2.3.5 Determination of total curcuminoid content. About 100 milligrams of the sample turmeric is accurately weighed into an extraction flask, then 30 milliliters of 95% ethanol is added and the mixture refluxed for 2½ hours. The content is cooled and filtered quantitatively into a 100-ml volumetric flask, thoroughly

washed and diluted to the mark with 95% ethanol. 10 ml of the extract is pipetted into a 100-ml volumetric flask and diluted to the volume with 95% ethanol. The absorbance of the extract is measured at 425 nm in a 1-cm cell against the 95% ethanol blank.

Accurately weigh 0.0500 gm of standard curcumin into a 500-ml volumetric flask and dilute to the mark with ethanol. Then a series of standard solution containing 1,2,4,8 and 10 ppm of curcumin are prepared from this stock solution by dilution, and the absorbance of these solutions are measured as described above. Plot the absorbance against the concentrations of curcumin, and then determine the total curcuminoid content of each sample by reference to the calibration graph.

3. Results and discussion

Table 8: Results of the analyses.

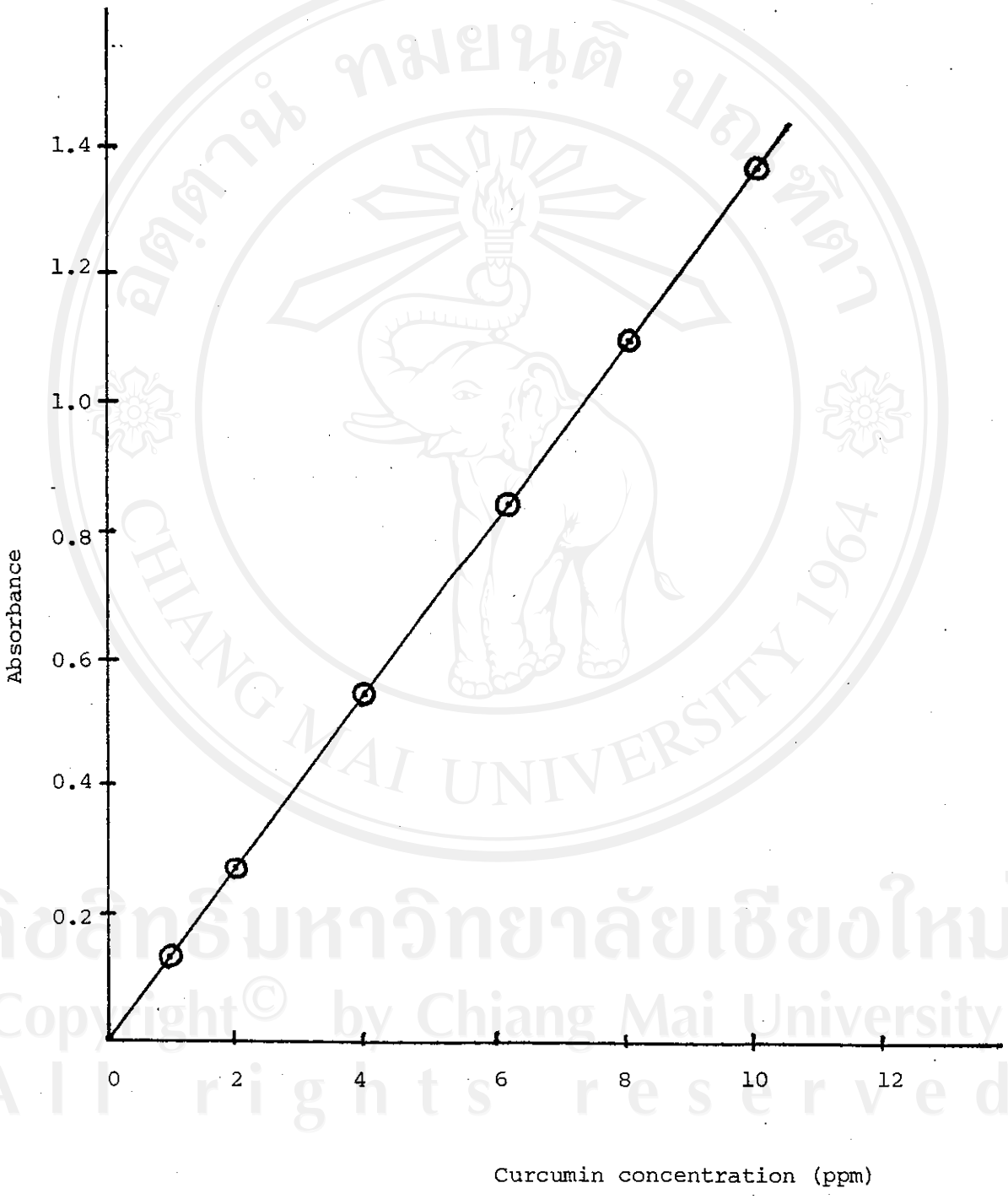
Details / Provinces	%Loss on* drying	%Volatile* oil content	%Total* ash content	%acid* insoluble ash	%Total* ether extract	%Nonvo* latile ether extract	%Total* curcuminoid content
ChiangMai	16.27	7.05	14.56	0.64	32.96	31.54	11.67
Yala	16.21	7.69	10.32	0.75	20.76	19.27	8.97
Ratchaburi	19.49	12.19	8.11	0.65	47.32	45.04	22.90
Lamphun	19.41	7.50	17.04	1.55	33.32	31.69	13.02
ChiangRai	17.04	8.92	6.54	0.42	20.58	19.22	8.78
SuphanBuri	15.59	7.22	8.41	0.67	13.18	11.77	7.46
Phayao	18.94	10.64	15.10	2.80	23.54	22.33	8.90
Nakhon Pathom	18.46	17.58	9.94	0.75	40.14	38.43	19.42
Chantha buri	14.91	11.22	10.37	0.72	29.81	26.40	12.48
Chonburi	14.99	7.91	12.09	0.65	15.93	14.53	5.75
Nakhonrat chasima	14.66	11.92	12.00	0.55	22.68	21.16	11.23
Phichit	9.13	9.50	9.57	0.64	14.79	13.57	6.05
Rayong	11.87	7.50	8.28	0.74	13.51	12.05	9.60
Songkhla	24.09	21.56	9.36	1.51	30.13	28.52	5.54
ChaiNat	15.41	12.32	11.20	0.93	16.36	14.34	7.62

*Average of 3 determinations of each sample

Table 9: Absorbance values of standard curcumin solutions

concentration of standard curcumin solution (ppm)	absorbance at 425 nm
1	0.138
2	0.276
4	0.550
8	1.100
10	1.370

Figure 5: Calibration curve of standard curcumin. All measurements were made at 425 nm by means of a spectrophotometer.



The results of analyses showed the following data;

Internal colour of turmeric rhizomes	yellow to orange brown
Loss on drying	9.13-24.09%
Volatile oil content	7.05-21.56%
Total ash content	6.54-17.04%
Acid-insoluble ash content	0.42-2.80%
Total ether-extractive content	13.18-47.32%
Non-volatile ether-extractive content	11.77-45.04%
Total curcuminoid content	5.54-22.90%

The most important criteria of the quality of turmeric are the volatile oil and the total pigment contents. The sample from Songkhla province contained exceptionally high content of volatile oil, 21.56%, while 8 of the samples contained 7-9% and 6 others contained over 10% of the volatile oil. The sample from Nakhon Pathom also showed very high percentage of volatile oil, 17.58%, and of Chai-Nat and Ratchaburi also contained more than 12%. Considering the volatile oil requirement of turmeric in the Indian Pharmacopeia of not less than 4.0%, the samples of turmeric in Thailand may be divided into 3 groups of high (7-9%), very high (10-13%) and exceptionally high (over 15%) volatile oil content.

The curcuminoid content also varied from 5.54% to 22.90%. Only two samples contained less than 6%, 7 samples between 6 to 10% 4 between 11 and 13%, and 2 of very high percentage of pigments, 19.42-22.90%. The study of turmeric in India showed 1.2-5.4% of curcuminoids, and the requirements of the U.S.Government Standard

was 5.0-6.6%, all expressed as curcumin.

The variations of quality occur from many factors, among which are the environmental conditions of cultivation (soil, nutrition, irrigation, light), maturity of the rhizomes, variety, and the method of preparation. Usually, turmeric produced for therapeutic use were harvested at 10 months old, or at the period of withering and drying of the vegetative parts. Some surplus would be left in the ground until next season to grow and propagation by themselves. The rhizomes of the latter would accumulate more of the ingredients. As C.Longa Linn is considered as a native plant, it endures the variation of climate and is rather free of plant diseases. Fertilizers are seldom used. Therefore, difference in the soil properties and yearly rainfall influences mainly the quality of the rhizomes.

As the results of analyses showed a wide range of the percentage contents, the lower limit obtained in the determinations of volatile oil, ether-extractives, and the curcuminoid contents would be accepted as the temporary standard of turmeric existed in the local markets. In case of the acid-insoluble ash content which signifies the dirt adhered to the crude drug, the maximum content obtained from 15 samples should be the not-exceeding limit. And in the case of loss on drying, the value includes moisture and the volatile substances contents, the latter of which is one of the criteria of quality of the drug, the range of the values should be retained as such.

For the temporary specifications and requirements for turmeric in Thailand, the followings are proposed.

Crude drug turmeric

Description and appearance	Dried mature rhizomes of *Curcuma longa* Linn., peeled or unpeeled, whole or sliced, yellow to brownish in colour
Internal colour	Yellow or golden yellow to orange brown
Loss on drying	9.13 to 24.09%
Volatile oil content	Not less than 7.0%
Total ash content	Not more than 17.04%
Acid-insoluble ash content	Not more than 2.80%
Total ether-extractive content	Not less than 13.0%
Non-volatile ether-extractive	Not less than 11.0%
Total curcuminoids content	Not less than 5.50%