CHEMICAL ANALYSIS OF SOME COMMERCIAL WINES

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (ANALYTICAL CHEMISTRY).

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JUNE, 1984.

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated and sources of information are specifically acknowledged by means of references.



Sylvester Y. Aboho

Date: 6th Oct. 1984

DEDICATION

TO

MY WIFE, MERNAN,

AND

MY CHILDREN

BEM, ILYAMBEE AND IER;

FOR THEIR

PATIENCE AND ENCOURAGEMENT

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisors, Drs. G.H. Ogbadu and O.A. Oyeleke, both of Biochemistry Department and Dr. H.T. Bozimo of Chemistry Department for their close supervision of this research work.

My appreciation also goes to the Chief Technologist, Mr. A.F. Smith and the entire technical staff for their various services rendered towards the fulfilment of this task.

I am also grateful to Messrs. S.A. Mom, E.A. Bature, James A. Akaa, Kahtan Alwan, Jonathan Chagu and Samuel Ajai, for their different contributions, that have in no small way, helped to see this work through.

ABSTRACT

Samples of five commercial wines were analysed for alcohol content (percent by volume), total sulphur dioxide, ash content and total acidity. Other parameters determined were ash alkalinity, tannins, pH and metal ions - potassium, sodium, magnesium, copper, iron, manganese, zinc, cadmium and lead.

Results of percent alcohol content, by volume gave higher values than their equivalents inscribed on the bottles in degrees proof.

Total sulphur dioxide for the various samples ranged from 129.61 + 0.49 to 260.99 ± 0.04 mg/l; and only White Lady (Samples E_1 and E_2) had total sulphur dioxide within the range stipulated by the United States Standards for Wines. Dubonnet (Samples D_1 and D_2) showed the lowest total sulphur dioxide content.

The amounts of tannins detected in all the wine samples were lower than previously published results. However, values were as expected higher for red or coloured wines than for white ones.

Cadmium was detected only in the tonic wines - Dubonnet and Ambassadeur, Apart from Sample E_1 (White Lady) lead content was found to be far in excess of the stipulated safe content.

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CHAPTER ONE

1.0 I N T R O D U C T I O N

1.1 WINES: WHAT THEY ARE

The term "wine", according to Herstain and Jacobs (1948) is such a broad one that when used without proper qualification includes a variety of products resulting from fermentation of both fruit and non-fruit materials. However, Hart (1971) described wines as fruit-based undistilled beverages, thus excluding all non-fruit beverages. Pearson (1976) also defined wines as products of fermentation of hexose sugars of fruits, usually grapes by yeast to form alcohol and carbon dioxide. Horne (1979) recently defined wines as alcoholic beverages obtained from the fermentation of freshly-gathered grapes the fermentation of which is carried out in the district where the grapes are grown and in accordance with the local tradition of the district. This means therefore that beverages made from fruits other than grapes are not wines. If they must be referred to as wines, their source materials have to be clearly stated, for instance as either elderberry, honey or blackberry wines in case of those made from elderberry, honey or blackberry respectively. This seems to be the most widely accepted definition. According to

Onitiri (1982), it had earlier been put forward by the Wine Trade Association. Onitiri (1982) further defined wines as fermented juice derived from the fruits of Vitis vinifera variety of the grape or the other important closely related varieties, Vitis labrusca and Vitis rotundifolia, as well as other less important ones. Wines are, therefore, grape-based undistilled alcoholic beverages, the fermentation of which is brought about by a special culture of yeast, usually Saccheromyces ellipsoidous. Where the grapes are crushed does not really matter. It entirely depends on how economical it is; either to crush where the grapes are grown or to transport the grapes to a winery away from their place of growth.

1.2 CLASSIFICATION OF WINES

There are as many types of wines as there are varieties of grapes and methods of wine making. In order that wines are easily identified, it is necessary that wines are classified. There are many and different criteria used in wine classification, and methods of classification also vary from one person, country or region to another. Generally, wines are classified according to place of production, colour, alcohol..

content, method of production and variety of fruit or grape (Kirk-Othmer, 1963). Herstain and Jacobs (1948), held the view that the basis of wine classification should also include the characteristics of the wine, as well as the use to which the wine is put. Pearson (1976) classified wines into three, namely table, sparkling and fortified wines; based on method of fermentation of the grape juice, characteristics, and use to which the wines are put. Others recognise only two main classes of wines, red and white, based on colour. Wines, according to Kirk-Othmer (1963), are said to be table when they contain below 14% (v/v) alcohol and desert when higher; and incorporating colour in classification gives white or red table or desert wines. There are cases where reference is also made to sweetness in wine classification. These are red or white table or desert wines that contain reasonable amount of sugar after fermentation.

1.3 MANUFACTURE OF WINE

The methods and processes involved in winemaking are in practice diverse but in principle the same. Adoption of any of these methods depends to some extent on the variety of the grape, the main ingredient of wine and the type and quality of wine in sight.

The main steps involved, apart from planting and training of the vine (Horne, 1979) include picking of the grapes, stemming or separation of the fruits from the stalks (for white wine), crushing and fermentation. Others are pressing, aging and secondary fermentation, with a number of slight variations, depending on the requirement of a particular winery. Given good climatic conditions, soil and proper cultivation and training of the vine, the grapes are usually ripe and ready for harvesting in autumn (Horne, 1979). In view of the fact that grape is the main ingredient of wine, its quality singularly influences that of the wine. In order, therefore, to obtain good wines, the stage of ripening of the grape among other things is closely monitored, before the fruits are harvested (Amerine and Ough, 1980, Hart, 1971). Kluba (1978), also pointed out that wines made without due regard to stages of ripening of the grape are not usually good. Having collected adequately riped grapes, they are stemmed and ready for crushing, so as to liberate the juice and get it in the form that can be inoculated with a selected culture of yeast. Methods of crushing vary from the old-fashioned hand

method to a modern highly mechanised one. The old Spanish method of crushing with feet is still being practised, but only at local levels (Horne, 1979). The must or unfermented grape juice is seperated from the skin, before sulphuring prior to inoculation and subsequent fermentation. Stemming and separation of must from the skin are very crucial stages in winemaking, since it is at these stages that a wine assumes its natural colour. It is also at these stages that the type and amount of fining agent is decided. Precisely, this is the time during which most of the phenolic compounds, including tannins, anthocyanins, catechins and other flavonoids found in wines are being extracted into the body of the wine. Some, however, are extracted during aging. Addition of sulphur dioxide is also very essential. It is a preservative, antiseptic in nature and inhibits the growth of molds and wild yeast, previously present on the grapes, before the must is inoculated with cultured yeast. pH and temperature are closely controlled at this stage. Fermentation takes place in containers called vats and lasts up to six weeks. Apart from transformation of grapes into alcohol and carbon dioxide, other minor changes including drastic fall in the acidity of the must

and protein content takes place. At the completion of fermentation, the new wine is transferred from the vats into wooden barrel for ageing, still with sulphur dioxide in the range of 75 - 150mg/l maintained throughout. The wine is also ready for fining and racking. The new wine can also at this stage be used as starting material for preparation of different types of distilled and undistilled beverages, such as brandies, fortified and sparkling wines. Fortified wines are those wines to which brandy has been added to stop fermentation and increase alcohol content (Lichine, 1977). These include Port, Sherry, and possibly Dubonnet and Ambassadeur. Sparkling wines are those made effervescent by the presence of carbon dioxide in the bottle, the best of which is Champagne.

1.4 NEED FOR WINE ANALYSIS.

Even though according to Thorpe (1937) full determination of all constituents of wine is rarely required, routine physical and chemical analysis of grape juice and wines is important and constitutes one of the most essential aspects of modern enological quality control in wineries (Amerine and Ough, 1969).

These analyses are directed toward checking the freedom of such wines from adulteration and manipulation (Thorpe, 1937). Analyses and checks in wineries right from the vineyard phase to the time the product is sold is very essential if consistency in the quality of the product is to be maintained (Peterson, 1974). Apart from quality control in the industry, analysis of wine is also essential in determining how to handle the must and wine right from the time of harvesting of the grapes to the final bottling of the product. Junge, (1976) also pointed out that apart from process and quality control, wine analysis helps in standardisation of analytical methods, detection of adulteration and preservation of analytical data for wines. Amerine and Ough (1969, 1980), summarised the importance of musts and wine analysis, which apart from reasons cited above, include strict compliance with legal limits imposed on some wine constituents such as preservatives and alcohol content. They also pointed out that knowledge of composition of wines is required both in blending and further use of the wine, for example in production of other beverages. In France and other European countries, prices are fixed on wines mainly on the basis of alcohol .

content and taxes collected are also determined by their specific gravity (Amerine and Ough, 1969, 1980).

Analysis of wines, as earlier mentioned, is not necessarily aimed at organoleptic quality evaluation; since this is better done by tasting. Almashi (1977) cited criteria for organoleptic quality of wines, which include both the kind and intensity of flavour, colour and clarity; these are quite different from what are most often looked for in analysis. The most important parameters determined in wine analysis among others include alcohol content, preservatives (of which sulphur dioxide seems the most important in winemaking), volatile and total titratable acids and extract. Others are reducing sugars, tannins or total phenolic compounds, colouring matter ash and ash alkalinity potassium acid tartrate, refractive index, pH and contaminants, which could either be organic, inorganic or both (Eynon, 1924 and Pearson, 1976). Contaminants could also be either those occuring naturally in wines, such as phenolic compounds or those picked by the grape from its surroundings or from the equipment in the course of winemaking (Gralhano, 1964).

Alcoholic content: This is mainly the ethyl alcohol, but a lot of other lower and higher alcohols have been found in wines. The amount of each of these lower alcohols and fusel oils in a given wine depends on the variety of the grape, extent and mode of fermentation (Amerine and Ough, 1980). Alcohols are derived directly from the fermentation of grape juice. Ethyl alcohol is said to be important due to the taste it imparts to the wine. It also adds little odour and "body" to it (Onitiri, 1982). Alcohol content of wines is closely regulated by law and is needed for tax assessment on wines.

Acidity: Acids are an important constituent of most preserved foods. According to Amerine and Ough (1980), there would be no commercial grape juice without acids. Grape juice and wine would taste flat and easily go bad without acids; which are mainly organic and include tartaric, malic and citric acids. Other acids found in wines are acetic, gluconic, oxalic, glycolic succinic and a few others. These acids are responsible for the sour taste of wines and without them fermentation would not proceed and produce satisfactory products, thus resulting in poor quality

wine. Close control and monitoring of acidity in wines and wineries is therefore very important.

pH: pH is said to influence the quality of wine more than does acidity (Amerine and Ough, 1980). Webber (1957), outlined the importance of pH in food and drug industries; which include its influence on microorganism growth, colour, taste redox potential and particularly for wines free-to-bound sulphur dioxide ratio. It has been shown by Procopio and Antona (1964) that low pH favours free sulphur dioxide presence in wine. A decrease, from pH 3.5 to pH 2.8 increases free sulphur dioxide five-fold (Procopio and Antona 1964). At pH 3.9 only 0.7% of the total sulphur dioxide is in free state, while 60% is found in the free state at pH 1.5. A very low pH stops fermentation completely. pH of commercial wines ranges between 2.8 and 3.8 (Weast 1975).

Ash and Ash alkalinity: Determination of ash content of a substance is a measure of the inorganic matter present in the substance. In wine analysis, knowledge of amount of ash and its alkalinity is very useful in some enological formulae designed to detect watering, sugar-addition to wine and other forms of

falsification, including use of unriped grapes, sugared and watered musts or free-run grape juice (Amerine and Ough, 1980). Amount of ash is also a distinguishing characteristic between red and white wines. Alkalinity of the ash, according to Amerine and Ough (1969) is a measure of organic acid salts present in the original wine. Its knowledge is important in making a balance between total organic ions in wines.

Preservatives: Chemical preservatives have been defined by Food and Drug Administration of America as Chemicals which when added to food tend to prevent or retard its deterioration (Rabach, 1980). These, however, do not include common salts, sugars, vinegars, spices or oils extracted from spices. Also excluded are substances added to food by direct exposure to wood smoke and chemicals applied for herbicidal or insecticidal purposes. There are about four major classes of preservatives; generally used in food industries. These include benzoates, parabenes, propionates and sorbates. Other minor but important preservatives include acidulants, nitrites and sulphites. Sulphites generally include sulphur dioxide, sulphite, bisulphite, and metabisulphite

salts. Sulphites are main fruit juice preservatives and most effective against yeast, molds and bacteria (Robach, 1980). They are important preservatives used in wines. Sulphur dioxide in liquid form, compressed sulphur dioxide in gas cylinders, metabisulphite or bisulphite salts could be added to wine or must at the time of crushing and its presence maintained throughout production. Total sulphur dioxide of between 50-100 ppm ensures a pure fermentation and inhibits enzymatic oxidative darkening of must and young wines (Kielhoefer, 1964). It is, therefore, very essential to monitor closely amount of both total and free sulphur dioxide in both musts and wines. Total and free sulphur dioxide have a legal minimum and maximum limits of 200 to 350 and 30 to 100 mg/l respectively.

Extract: This is a measure of soluble solids in the wine. It is an indication of the sugar content in the original must. Extract-to-alcohol ratio is a very important factor in determining falsification of wines. Eynon (1924) observed that genuine red wines do not have an extract-to-alcohol ratio higher than 4.5 and that of white wine does not exceed 6.4. Higher values are a proof of added alcohol.

Tannins: Tannins and other phenolic compounds are considered indispensible constituents of grape wines, irrespective of class, colour or origin. Most often however, deep red wines contain higher amounts of tannins and other phenolic substances than white ones; and the main difference between white and red wines, apart from colour is due to differences in amount of tannins and anthocyanins. Tayleur (1973), observed that wines without tannins would be dull and insipid, an effect almost equal to that due to absence of acids. Catechins and other flavonoids are found along with tannins and anthocyanins in wines. They are extracted mainly from seeds, skins and stems and are said to be antiseptic and contribute astringent flavour to wines (Singleton, 1974). These phenolic compounds are also very important during aging and finishing of wines. There are two distinct groups of tannins - the hydrolyzable and condensed tannins (Singleton and Kratzer, 1973). All these have protein binding and leather forming properties, but differ significantly in occurance and breakdown products. Tannins are toxic and even fatal when 3 - 5% of it finds its way into the blood stream or other vital organs like the liver and kidney. (Singleton and Kratzer, 1973). Toxicity of these

phonolic compounds is mainly due to their ability to precipitate proteins of the body (Singleton and Kratzer, 1973). Some are also carcinogenic (Singleton and Kratzer, 1973). Determination of tannins will make it possible to maintain amount of tannins and other phenolic compounds, within limits of human tolerance.

Metal contaminants and mineral nutrients: Inorganic matter of varied range including essential nutrients and metal contaminants usually form a significant part of our food. They get into food through various routes and means. These include absorption of the ions in solution in the soil, from irrigation water and the atmosphere by the leaves, stems and fruits. Other routes are through processing equipment, containers and packaging materials. Each of these inorganic elements has its definite function in the body system. Some are toxic and thus called contaminants, others essential and known as nutrients. Potassium, sodium, calcium and magnesium are found in large amounts in wines (and other foods) as shown by Meuron (1963). They are therefore known as a group of essential macronutrients. Cobalt, copper, iron, manganese and zinc are essential trace metals.

Aluminium, boron, chromium, nickel and tin are non-nutrional and non-toxic elements. Arsenic, lead, mercury, cadmium, antimony and fluorine are very well known toxic trace elements, their presence in food is therefore termed contamination. Metal determination in wines has been done by various groups of analysts. Basile (1975) observed that wines produced from vineyards along roads and motor-ways contain high amounts of lead, exceeding the state (Italy) limits. Nagy et al (1976) found that 94.12% of the 560 wines studied contained less than 0.4 mg/l of lead, 2.67% had between 0.4 to 0.6mg/l and 3.21% contained more than 0.6 mg/l. Mordi et al (1976) gave results of metal determinations in Italian wines as shown in Table 1.1.

TABLE 1.1

TRACE METALS IN ITALIAN WINES

Metal	Concentration, mg/l Maximum	Minimum
Cd	0.023	0.007
Cu	2.653	0.34
Fe	30.45	8.46
Pb	1.09	0.26
Zn	7.381	2.082

Apart from toxic effect of some of these metals, almost all of them cause colour problems in wine when appreciably high in concentration. Potassium if high, precipitates out as potassium tartrate and it has been found that the corresponding sodium salt is less difficult to handle since sodium salts are more soluble than potassium salts (Thouks, 1974). Monier-Williams (1950) observed that when concentration of zinc in wines is high, it tends to react with sulphur dioxide giving off hydrogen sulphide, which in turn reacts with more sulphur dioxide, consequently precipitating the elemental sulphur, thus causing turbidity. Precipitation occurs in cases of aluminium, iron and tin. Various causes of wine turbidity have been identified, the root cause of which is lack of stability on the part of the wine itself (Thouks, 1974). Frenne (1976) named bacteria, protein/protein-metal complexes and metal tartrates as causes of turbidity. Other turbidity causing agents in wines are proteins, pectins, pentosans, galactose and other colloidal matter derived from grapes and yeasts (Usseglio-Tomasset, 1964). Thorough knowledge of interaction of metals in solution with sugars and other wine constituents will be of importance to wine makers in improving on the aesthetic quality of wines.

1.5 USES OF WINES.

Apart from being a source of revenue to the country that produces and exports wines, Lichine (1977) outlined five principal uses of wine to human beings. These are, as food, medicine, and antiseptics for both external and internal use. Wines are also very important in weight reduction and as appetizers.

Wine as food: In many countries of the world, wines are taken alcng with meals, thus serving as both food and appetizers. According to Lichine (1977) these wines taken with food and the food itself taste better than if each of them is taken seperately. A given mass of wine is capable of generating as much energy as an equal mass of carbohydrate. Wines contain vitamins A, B and C and all the thirteen mineral nutrients - calcium, phosphorus, magnesium, potassium, chlorine, sodium, sulphur, iron, cobalt, copper, iodine, zinc and manganese. Wines contain significant amounts of protein and about 95% alcohol is readily converted for immediate use.

Wine as medicine: Some wines are rich in spices and/or drugs and as such have long been used as medicine. Wines (Lichine, 1977) are used just as a general tonic or as specific medicine in the cure of different kinds of ailments. They are also indispensible in low-sodium diets and are said to reduce chances of developing gallstone (Linchine, 1977). Those rich in iron conteract iron defficiency in anemia.

Wines in weight reduction: When wines are taken into the body, they are oxidised like carbohydrate, but unlike carbohydrate they generate energy but leave virtually nothing solid in the body, thus causing no weight increase. Wines can, therefore, be used effectively to supply energy needed by the body to work, without the individual gaining additional weight.

Wines as appetizers: Wines, especially the red dry ones are capable of exciting the olfactory nerve and the taste buds thereby sharpening sluggish appetite (Lichine, 1977). Heavy concentration of wines, however inhibits appetite, but in dilute forms as they are usually taken, prompt the flow of saliva,

hydrochloric acid and gastric secretion thus starting hunger and appitite in action.

Wines as antiseptics: Wines and alcohols have always been used as antiseptics. Red wines are capable of killing Staphylococus aureus and other bacteria (Lichine, 1977). Wines are well known in treatment of bronchitis and influenza.

1.6 AIMS AND OBJECTIVES.

It has been established that wines are the most adulterated foods in the world (Liverseege, 1932). This may have been due to the loose manner the word "wine" is being used, or due to the availability of a wide range of starting materials. Probably adulteration of wine is possible because the most important phenomenon in winemaking - fermentation - occurs naturally and therefore winemaking is within reach of almost everybody. It could be recalled that, Zulfikar Ghose once said that there is no quicker way to make cash than by illicit liquor (Tayleur, 1973).

In order, therefore, that poor quality wines, susceptible to all kinds of microbial attacks due

to lack of proper preservation and good production techniques do not find their way into the hands of consumers, it is very essential that wines offered for sale are subjected to thorough analysis. Any wines found to be highly adulterated and possibly toxic should be withdrawn from circulation. It is in the light of these important facts that this work has become necessary. This project, therefore, intends to determine some parameters of various important commercial wines on sale in the market, with a view to assessing their level of adulteration vis-a-vis their quality and standard as compared with those obtained in other parts of the world.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 SAMPLES AND SAMPLING.

Two bottles each of five different commercial wines were analysed. The samples were designated by letter A to E. Their full description as inscribed on the bottles is as entered in Table 2.1.

TABLE 2.1

DESCRIPTION OF SAMPLES ANALYSED (AS INSCRIBED ON THE BOTTLE).

Sample designatior	Commercial name	Alcoholic strength	Colour (visual)	Other Particulars
A_1 and A_2	Dark Lady	15^{o}	red	A prenium dry wine, exported by Robert Misner KG; West Germany. Bottled By Ozde Distilleries **Limited** Lagos. Cork stoppered
B_1 and B_2	mbassadeur	16^{o}	(Deep red)	A tonic wine, made in France, and bottled in Nigeria, by Nigerian Distilleries. Metal Screw Capped.

Table 2.1 (cont'd)

Sample designation	Commercial name	Alcoholic strength	Colour (visual)	Other Particulars.
C_1 and C_2	Rose Lady	15^{o}	red	Same as A_1 and A_2
D_1 and D_2	Dubonnet	18^{o}	(deep red)	Tonic wine, made in France, and bottled in Nigeria, by Nigerian Distilleries. Contains natural sugars, calcium salt, natural vitamins B and C. Metal Screw capped.
E_1 and E_2	White Lady	15^{o}	Orange	A delicate wine. Exported by Robert Misner KG; West Germany and bottled by Ozde Distilleries. Cork stoppered.

Samples were bought in whole bottles from a number of stores in Samaru and Sabon Gari markets of Zaria. Attempts were made not to buy all the two samples of any one wine from the same shop. Samples were exposed as little as possible to the atmosphere. Since some bottles were cork-stoppered, a working portion of each sample was transferred into a clean screw capped sample bottle and both the bottles of wine and sample bottles tightly stoppered. Further, in an attempt to retain all or most of the wine constituents, especially volatiles, the mouths of the wine bottles were wrapped with aluminium foil. The sample bottles were refilled as regularly as they were used up.

2.2 EXPERIMENTAL

2.2.1 pH MEASUREMENT

APPARATUS: Griffin Battery type pH meter with combined reference electrode was used.

REAGENTS: Standard buffer solutions of pH 7 and 4 were prepared by adding a satchet of the pH hydrion buffer of specific pH to a specified volume of distilled water.

PROCEDURE:

The meter was first cleaned and battery tested. The combined reference electrode, which had for some time been dipped in 0.1M hydrochloric acid was thoroughly rinsed with distilled water and then connected to the meter; and meter calibrated with standard buffer solutions. The pH of the samples were measured by simply dipping in the electrode, allowing enough time for equilibrium to be attained and taking the readings, from the scale.

2.2.2 ACIDITY DETERMINATION.

The AOAC (1980) method was used for total acidity determination.

CHEMICALS:

(i) 0.1M sodium hydroxide solution

(ii) 1% phenolphthalein indicator solution.

PROCEDURE

200 ml of hot distilled water was titrated to phenolphthalein end-point with 0.1M sodium hydroxide solution. 5 ml of the sample was added to the hot water, mixed thoroughly and further titrated with the same sodium hydroxide solution to the same end-point.

The acidity, expressed as tartaric acid was calculated from this formula:

$$\text{Total acidity, g/100ml} = \frac{(V)(M)(75)(100)}{(1000)\quad(v)}$$

where,

V = volume of sodium hydroxide solution, in ml.

M = molarity of sodium hydroxide solution.

v = volume of sample, in ml.

2.2.3 TOTAL SULPHUR DIOXIDE.

Accurate determination of total sulphur dioxide requires taking cognizance of the presence of other volatile substances in wines such as aldehyde which usually combine with sulphur dioxide (Burrough and Spark, 1963). The AOAC (1980) iodimetric method was employed in the determination.

REAGENTS.

(i) 0.02M sodium thiosulphate solution, standardized against potassium iodate solution.

(ii) 0.02M iodine solution, standardised against standard sodium thiosulphate solution.

(iii) 1M sodium hydroxide solution

(iv) 1:3 (about 33% v/v) sulphuric acid

(v) 1% starch indicator solution

(vi) Anhydrous sodium bicarbonate.

PROCEDURE:

25ml of the sample and 25ml of 1M sodium hydroxide solution were pipetted into a 250-ml Erlenmeyer flask, and the two solutions thoroughly mixed. The flask was set aside for about ten minutes. A pinch of anhydrous sodium bicarbonate was added, followed by 10ml of 1:3 (about 33% v/v) sulpuric acid. 25 ml of 0.02M iodine solution was added to the flask and the excess backtitrated with standard sodium thiosulphate solution. Starch indicator was added after most of the iodine had been titrated, and solution finally titrated to the end-point, which corresponds with the disappearance of the blue starch-iodine colour.

$$\text{Total sulphur dioxide, mg/l} = \frac{[(V_1M_1)-(V_2\ M_2)](64)(1000)}{V}$$

where

V_1 = volume of iodine solution added, in ml,

M_1 = molarity of iodine solution added

V_2 = volume of sodium thiosulphate solution in ml.

M_2 = molarity of sodium thiosulphate solution.

v = volume of sample used; in ml

2.2.4 ALCOHOLIC CONTENT DETERMINATION:

Specific gravity method (Amerine and Ough, 1969, Anon, 1968, AOAC, 1980) was used, the method which according to Leach and Korcoses (1976) has advantages over others, both in terms of time taken to complete the determination and precision. The same method was used for determination of specific gravity of wine from which extract was calculated; from:

$$dr = dw - da + 1.000$$

where,

dr = wine residue

dw = specific gravity of wine

da = specific gravity of alcohol-water mixture.

APPARATUS:

(i) Specific gravity bottle (Pycnometer)

(ii) Mettler Analytical balance, Type H15

(iii) A Haak dual prupose thermostatically controlled electric water bath, carrying a zeal thermometer, maintained at 20 $\pm$ 1°C.

PROCEDURE:

(a) Determination of mo(Mass of the empty specific gravity bottle): A 50-ml specific gravity bottle soaked overnight in chromic acid was thoroughly washed and dried at about 100°C, in an oven. It was taken into the balance room in a heat resistant desicator, and allowed to attain balance room temperature. It was finally weighed and the mass, mo, recorded.

(b) Determination of mw (mass of water content of the specific gravity bottle): The empty specific gravity bottle was filled with distilled water, stoppered and placed in a water bath maintained at 20 $\pm$ 1°C for about an hour. It was removed, external water dried with soft tissues and the bottle, with water taken on a non-conducting clean cardboard paper

to the balance room and allowed to attain balance room temperature. It was weighed and the mass, mw, obtained by subtraction.

(c) Determination of specific gravity $20^{\circ}/20^{\circ}C$: 100ml of the sample, in a volumetric flask was transferred into a round-bottomed distilling flask with rinsing. The wine was distilled, and the same volumetric flask used to collect distillate up to the 100ml mark. The specific gravity of the ethanol-water mixture was determined, in a similar way as mw. The specific gravity of the undistilled wine sample was also determined, at $20 \pm 1^{\circ}C$.

(d) Calculation of specific gravity of alcohol-water mixture: Based on Anon's (1968) recommendations, specific gravity, $D20^{\circ}/20^{\circ}C$ has to be calculated using equation (1).

$$D20/20^{\circ}C = \frac{1}{mw}\left[Pa - mo * d_i\left(\frac{Pa}{db} - \frac{mo}{dw}\right)\right] + \frac{d_i}{dw} \qquad \ldots(1)$$

where,

$D20^{\circ}/20^{\circ}C$ = specific gravity at $20^{\circ}C$

mw = mass of water content of the specific gravity bottle at the measuring temperature.

Pa = mass of specific gravity bottle filled with the sample

mo = mass of empty specific gravity bottle.

db = density of the weights (= 8.4g/cm^3)

d_i = density of air under weighing conditions when the specific gravity bottle is filled with the alcohol-water solution and it is equal to 0.001053.

dw = relative density of water measured as g/cm^3 at 20°C (= 0.99823)

dg = density of glass (= 2.32g/cm^3)

Equation (1) can further be reduced to equation (2)

$$D20^{\circ}/20^{\circ}C = \frac{1}{mw}\left[Pa - mo + d_i\ (Vp - \frac{Pa}{db})\right] \qquad ...(2)$$

$$Vp = \frac{mo}{dg} + \frac{mw}{dw}$$

Equation (2) was used to calculate the specific gravity of the ethanol-water solution of the samples and reference made to Appendix 1, to obtaine percent alcohol content by volume. Extract, g/100ml was also obtained from Appendix 2.

2.2.5 ASH CONTENT DETERMINATION.

The AOAC (1980) method was used.

APPARATUS:

(i) Muffle furnace - Carbolite model
(ii) Gallenkamp hotplate
(iii) Infrared lamp
(iv) Porcelain crucibles
(v) Heat resistance desicator.

PROCEDURE:

Ten 50-ml porcelain crucibles were thoroughly washed with chromic acid and rinsed with distilled water. They were dried to constant weight (m_1) in a muffle furnace at 600°C and allowed to cool in a desicator. 25ml of the samples were pipetted into each of the crucibles and evaporated to dryness. Infrared lamps were used to effect complete evaporation to dryness in case of the tonic wines. The residue were incinerated with a Bunsen burner and ash finally ignited in a muffle furnace to grey or whitish coloured ash at 525°C. Black coloured ash were treated with a few drops of distilled water, dried in an oven and reheated in the furnace. The ash was cooled in a desicator and weighed (m_2).

$$\text{Ash content, g/100ml} = \frac{M_2 - m_1}{v} \times 100$$

where v is volume of sample, in ml.

2.2.6 DETERMINATION OF ASH ALKALINITY.

Ash alkalinity was determined titrimetrically. Due to the difficulty in detecting the methyl orange indicator end point, as a result of small volumes of acid usually required for neutralisation, the AOAC (1980) back titration method was used, instead.

REAGENTS:

(i) 0.1M sulphuric acid solution
(ii) 0.1M sodium hydroxide solution
(iii) 1% methyl orange indicator solution.

PROCEDURE:

Ash was obtained in a similar way as in ash content determination. The porcelain crucible with ash was placed in a 400-ml wide mouth beaker and a few drops of water added, followed by 25ml of 0.1M sulphuric acid solution. The beaker was covered with a clean watch glass and heated slightly

on a wath-bath with stiring. The watch glass was rinsed thoroughly with a few drops of distilled water, five drops of 1% methyl orange indicator solution added and the solution titrated to the end-point.

2.2.7 METAL CONTAMINANTS AND MINERAL NUTRIENTS.

Atomic absorption spectrophotometry was used for all other metals, apart from sodium and potassium, which were determined by the use of direct reading flame photometry.

REAGENTS:

(i) Deionised water

(ii) Stock solutions of all metals (preperation shown in Appendix 7).

(iii) 50/50 (v/v) hydrochloric acid solution

(iv) Lanthanum solution

(v) Calcium solution

APPARATUS:

(i) Pye Unicam SP 1900 Atomic Absorption Spectrophotometer, Air-acetylene gas.

(ii) Corning EEL flame photometer.

PROCEDURE:

Sample preparation: The ash was washed with 10ml of deionised water into a 50-ml beaker and 10ml of 50/50 (v/v) hydrochloric acid added. The suspension was boiled briefly on a Gallenkamp hotplate, filtered into a 100-ml volumetric flask and made up to the mark with deionised water.

Determination: The standard solutions (of the metals to be determined) were aspirated first, then the samples. The standards were repeated after all the samples were aspirated. Average absorbance of the standard solutions were plotted against concentration in ppm. From the calibration curves (appendices 2a - 2d), concentrations of lead, magnesium, copper, manganese, iron, cadmium and zinc were determined.

The highest sodium and potassium standards were used to set the maximum percent emission intensity of the instrument, before lower standards were aspirated. From the calibration curves (Appendix 2f) amounts of potassium and sodium in the samples were determined.

2.2.8 DETERMINATION OF TANNINS.

The AOAC (1980) method was used despite Objections (Singleton, 1974) that Folin-Denis reagent is prone to precipitation problems.

APPARATUS:

Bausch and Lomb Spectronic 20, Phys. Chem. No. 2, with CE-A59RX Phototube was used at 760nm wavelenght.

REAGENTS:

(i) Folin-Denis: To 138ml of distilled water in a 500-ml round-bottomed flask, 25g of sodium tungstate ($Na_2WO_4.2H_2O$), 5g of phosphomolybdic acid ($H_3PO_4.12MoO_3.24H_2O$) and 12.5ml phosphoric acid (H_3PO_4) were added. The mixture was reflux for two hours, cooled transferred into 250-ml volumetric flask and diluted to the mark with distilled water.

(ii) Saturated sodium carbonate solution: 35g of anhydrous sodium carbonate were each dissolved in three different 100-ml volumetric flask at about 75°C by heating on a Gallenkamp hotplate. The solutions were set to cool overnight and then seeded with a crystal of sodium carbonate decahydrate ($Na_2CO_3.10H_2O$); and filtered through glass wool.

(iii) Tannic acid stock solution: 1.000g of analytical grade tannic acid was dissolved in one litre of distilled water, and from it intermediate standard (100mg/l) and working standards were prepared.

DETERMINATION:

Series of working standards ranging from 0 to 12ppm (with 0 ppm as blank) were prepared from the intermediate standard by pipetting 0 ml, 2ml, 4ml 6ml, 8ml, 10ml and 12ml into seven different 100-ml volumetric flasks; containing about 75ml of distilled water. To each of these flasks 5ml of Folin-Denis reagent and 10ml of saturated sodium carbonate solution were added, strictly in that order and solutions made up to the mark. The solutions were set aside for about thirty minutes for colour to develop. 1ml of each sample was pipetted into ten different 100-ml volumetric flasks and treated similarly as the standards. Samples and standards were analysed using spectronic 20 and amounts of tannins as tannic acid read from the calibration curve (Appendix 2g).

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

The results of percent alcohol content (v/v), pH, ash content, extract and alcohol-to-extract ratio are shown in Table 3.1. Those of total acidity, total sulphur dioxide, ash alkalinity and tannis in Table 3.2 and results of metal ions in Tables 3.3 and 3.4.

TABLE 3.1

ALCOHOL, ASH AND EXTRACT CONTENT OF WINE SAMPLES

Sample	% Alcohol[a] (v/v)	pH	Ash content g/100ml	Extract[a] g/100ml	Extract-alcohol-ratio
A_1	15.67 ± 0.32	3.38 ± 0.029	0.233 ± 0.006	2.58	6.07
A_2	14.36 ± 0.41	3.40 ± 0.050	0.233 ± 0.005	2.32	6.19
B_1	15.67 ± 0.32	3.15 ± 0.050	0.212 ± 0.007	15.89	0.99
B_2	15.21 ± 0.24	3.28 ± 0.029	0.217 ± 0.006	15.89	0.96
C_1	13.98 ± 0.28	3.41 ± 0.078	0.213 ± 0.009	2.06	6.71
C_2	13.43 ± 0.11	3.38 ± 0.028	0.213 ± 0.009	1.80	7.46
D_1	17.23 ± 0.23	3.30 ± 0.100	0.192 ± 0.004	16.12	1.07
D_2	17.32 ± 0.24	3.32 ± 0.029	0.197 ± 0.003	16.38	1.06
E_1	13.89 ± 0.27	3.17 ± 0.080	0.189 ± 0.014	1.80	7.72
E_2	13.26 ± 0.14	3.18 ± 0.029	0.175 ± 0.003	1.80	7.37

Mean of triplicate determinations

± Standard deviation

a = means of duplicate determinations

3.1 ALCOHOL CONTENT

The percent alcohol content (v/v) (Table 3.1) varies from a maximum value of 17.32 ± 0.24 to a minimum value of 13.26 ± 0.14, with Dubonnet (samples D_1 and D_2) and White Lady (samples E_1 and E_2) showing the highest and lowest percent alcohol content respectively. Going by either the United States or United Kingdom System, percent alcohol content by volume is supposed to be about half alcoholic strenght in degrees proof (Tayleur, 1973); usually inscribed on the bottle. However, alcohol content by volume, found through analysis is almost equal to that given in degrees proof. If this high alcohol content (v/v) observed in the samples is not due to some chemical reactions, for example reduction of some carbonyl compounds especially aldehydes in the wines to alcohol, it could mean that the manufacturers have simply decided to give lower values in degrees proof, while the actual alcoholic streng is much higher. Alternatively it could be that to the manufacturers', (Ozde and Nigerian Distilleries) alcoholic streng in percent by volume and in degrees proof mean exactly the same in terms of alcoholic strength. The last option seems to be the case with Dubonnet (samples D_1 and D_2) where alcoholic strength is given in both degrees proof and percent by volume.

Here the alcohol content by volume, found through analysis was almost equal to alcoholic strength in degrees proof, in scribed on the bottle.

When alcohol content, by volume found through analysis is compared with results of previous works and American Standards it will be found that despite the differences between the experimental values and those given in degrees proof, the alcohol content of the wines does not lie beyond the acceptable legal ranges (Amerine and Ough, 1980). Dubonnet (Samples D_1 and D_2), with its high alcohol content of between 17.23 $\pm$ 0.23 and 17.32 $\pm$ 0.24 shows that the wine is probably a fortified or sweet wine (Amerine and Ough, 1980, Herstain and Jacobs, 1948). On the basis of alcohol content, it could also be said that all other wines are likely table wines - either dry red or white (Amerine and Ough, 1980); or even sparkling wine, especially Rose Lady (samples C_1 and C_2), with its average percent alcohol content of 13.71 $\pm$ 0.20. It is not easy to say precisely to which class a particular wine belongs on the basis of its alcohol content, due to the wide variation in alcohol content even within a particular class of wine (Amerine and Ough, 1969, 1980; Herstain and Jacobs, 1948; Kirk-Other, 1963; Thorpe, 1937). The result of the alcohol

content determination (Table 3.1) shows also that all the wines contain alcohol much higher than the minimum 10% that would otherwise render them susceptible to microbial growth (Amerine, 1974). The wines could, therefore, on the basis of alcohol content be said to be safe and free from spoilage and adulteration. Variations in alcohol content between samples of the same wine-type do not also lie beyond acceptable limits for a class of wine, so as to cast doubt as regards the genuineness of one of the samples.

3.2 pH.

pH value of the wines lie between a maximum and minimum value of 3.41 ± 0.078 and 3.15 ± 0.050, which are within the specified limits (Weast, 1975). Since an optimum pH ensures adequate sulphur dioxide in the free form (Procopio and Antona, 1974), it could be said, on the basis of pH, that the wines are safe and free from microbial attack and subsequent spoilage.

3.3 ASH CONTENT

The result of ash content determination (Table 3.1) shows with the exception of Dubonnet (Samples D_1 and D_2) lower values for the white than red wines. Ash content of genuine wines usually lies between 0.2 and 0.3g/100ml,

even though ash contents as low as 0.11g/100ml and as high as 0.44g/100ml are sometimes encountered (Eynon, 1924). However, values lower than 0.11g/100ml are clear indication of adulteration, especially due to watering of the must. Low ash contents of between 0.192 $\pm$ 0.004 to 0.197 $\pm$ 0.003 g/100ml for Dubonnet (samples D_1 and D_2), a deep red coloured wine, therefore suggests watering and artificial colouring of the wine. Ash contents of other samples are within stipulated limits, with White Lady (Samples E_1 and E_2), a white wine having the usual lower ash content than the other red and rose coloured wines.

3.4 EXTRACT.

Extract determination (Table 3.1) has pointed out clearly, with its higher values for the tonic than Ozde bottled wines that Dubonnet and Ambassadeur are certainly sweet wines. High extract values imply high sugar content, since it is a measure of sugar content of the original must (Amerine and Ough, 1980). It is, however, the alcohol-to-extract ratio that is of interest to wine analysts, when it comes to distinguishing between genuine and adulterated wines (Onitiri, 1982). It is said (Eynon, 1924) that alcohol-to-extract ratios of genuine red wines hardly exceed 4.5,

while those of white wines may be as high as 6.5, but never higher. The result has shown alcohol-to-extract values within acceptable ranges for genuine wines in the case of Dubonnet (Samples D_1 and D_2) and Ambassadeur (Samples B_1 and B_2), but not for the Ozde bottled wines - that is Dark Lady (samples A_1 and A_2), Rose Lady (samples C_1 and C_2) and White Lady (samples E_1 and E_2). High values of alcohol-to-extract ratios, for these wines, especially Rose Lady (samples C_1 and C_2) and White Lady (samples E_1 and E_2) suggest alcohol addition. This fact had earlier been observed through percent alcohol (v/v) where it was found that it far exceeded alcoholic strenght in degrees proof, inscribed on the bottles. The extremely high extract values for Dubonnet (samples D_1 and D_2) and Ambassadeur (samples B_1 and B_2) is also an indication of sugar addition, probably designed to sweeten the wines.

TABLE 3.2

Sample	Total acidity g/100ml	Ash Alkalinity ml of 0.1M H_2SO_4 for 100ml of sample	Total SO_2 [b] mg/l	Tannins [b] mg/100ml
A_1	0.492 ± 0.010	15.78 ± 0.051	146.69 ± 0.90	85 ± 2
A_2	0.513 ± 0.011	16.44 ± 0.064	142.18 ± 1.10	87 ± 3
B_1	0.540 ± 0.030	9.95 ± 0.045	166.69 ± 0.49	89 ± 0
B_2	0.552 ± 0.050	9.95 ± 0.029	146.69 ± 0.49	89 ± 2
C_1	0.572 ± 0.054	15.41 ± 0.013	239.10 ± 1.46	41 ± 1
C_2	0.557 ± 0.001	15.62 ± 0.022	243.97 ± 0.49	43 ± 1
D_1	0.544 ± 0.051	10.13 ± 0.045	131.92 ± 0.71	96 ± 0
D_2	0.547 ± 0.016	9.79 ± 0.023	129.66 ± 0.49	96 ± 1.5
E_1	0.531 ± 0.007	13.18 ± 0.035	260.99 ± 0.04	23 ± 0
E_2	0.523 ± 0.050	12.89 ± 0.050	229.34 ± 0.00	23 ± 0

Mean of triplicate determinations

± = stamdard deviation

b = means of duplicate determinations

3.5 TOTAL ACIDITY

Total acidity (Table 3.2) shows a fairly consistent result, with all the samples having nearly equal amount of total acidity. When compared with total acidity (in g/100 ml of dry red = 0.638; dry white = 0.604; light sweet white = 0.586; light sweet red = 0.590; fortified = 0.437; Sparkling = 0.619) reported by Guymon (1973), it will be found that results of this analysis compare favourably well with those of previous works. The same could be said when compared with results of Willey and Marsh and Joslyn, reported by Herstain and Jacobs (1948). Total acidity cannot, however, be used readily as a means of identification of classes to which wines belong due to its wide variation even within a particular class of wine (Amerine, 1974; Amerine and Ough, 1980). Dry table wines generally have total acidity ranging from 0.6 - 0.9 g/100ml and sweet and desert wines in the range of 0.4 - 0.65g/100ml (Amerine and Ough, 1980). Since none of the results lies outside this range, the wines could be said to be free from either watering (of the must) or other forms of adulterations that are detectable through total acidity.

129.61 ± 0.49 mg/l. Despite the fact that Dark Lady (samples A_1 and A_2), Rose Lady (samples C_1 and C_2) and white Lady (samples E_1 and E_2) are from the same source (Ozde Distilleries Limited), and the same class of wine, Dark Lady has a total sulphur dioxide content much lower than the others (Table 3.2). It appears that of all the wines analysed, only Rose Lady (sample C_1 and C_2) and White Lady (samples E_1 and E_2) gave total sulphur dioxide content that is within the legal maximum total sulphur dioxide of 200 - 300 mg/l (Amerine and Ough, 1969; 1980; Amerine, 1974). Since the antiseptic free sulphur dioxide was not determined it would be difficult to predict the effect of the generally low total sulphur dioxide on the quality of the wines. It has also been made more complex, since combined preservatives can as well be used in wine or food preservation (Popov, 1964; Robach, 1980). The low amount of total sulphur dioxide could thus be attributed to the use of combined preservatives. But since there is no rigid minimum limit in amount of total sulphur dioxide in wine and the amount of free sulphur dioxide is also dependent on pH and temperature (Procopio and Antona, 1964), the low amount of total sulphur dioxide would not obviously render the wines liable to microorganism attack; nor does it indicate that the wines are adulterated.

3.8 TANNINS.

The amount of tannins in the wines range from a maximum of 96 mg/100ml for Dubonnet (samples D_1 and D_2) to a minimum of 23 mg/100ml for White Lady (samples E_1 and E_2). Results of previous analyses showed a generally higher amounts of Tannins than have been found in the wines. Direct comparison of results with those of the previous workers is, however, not justified, due to variations in origin and variety of the grapes. The low values are nevertheless not unexpected (Singleton, 1974), since there has been changes in the method of cooperage; with many wineries now using stainless steel containers instead of the old fashioned wooden barrels. Development of new fining agents such as skimmed milk has also contributed in lowering amounts of tannins in wines. (Singleton, 1974). It is now possible to clearify wines, through fining without necessarily having to ensure that there is an optimum amount of tannins in the wines. Singleton and Kratzer (1973) have also shown that tannins are carcinogenic and this may also have been a contributing factor for the low amounts of tannins in the wines.

3.9 MINERAL NUTRIENTS AND METAL CONTAMINANTS

The determination of cations shows (Table 3.3 and 3.4) that, with the exception of manganese, all other metal investigated are present. The high potassium and sodium content is expected, bearing in mind that most preservatives are complex salts of these metals; for example, propionates and sorbates of sodium and potassium. In comparing these results with those of Amerine and Ough (1969) shows higher values for sodium than those reported by these authors. Their results on the other hand showed higher potassium contents than those found in the wines analysed. Recent results by Paul and Southgate (1978) showed higher amounts of these metals than were found in these wines. The general low amount of potassium as compared to results of Amerine and Ough (1969) and Paul and Southgate (1978) could probable be due to the fact that, as a result of constant problems of potassium tartrate precipitation, which causes turbidity (Thouks, 1974); some wineries may have preferred the use of sodium-based preservatives rather than those of potassium.

TABLE 3.3

MINERAL NUTRIENTS AND TRACE METALS IN WINE SAMPLES

Sample	mg/l						
	K	Na	Mg	Cu	Fe	Mn	Zn
A_1	440	88	24.8	1.0	7.2	ND	1.0
A_2	400	80	23.0	1.0	3.6	ND	1.5
B_1	320	82.8	21.1	ND	9.0	ND	0.84
B_2	280	160	16.3	3.2	7.2	ND	0.50
C_1	440	86.4	19.5	0.3	5.6	ND	0.42
C_2	480	72	21.2	1.0	7.2	ND	2.6
D_1	240	112	23.0	ND	8.8	ND	0.64
D_2	320	108	21.1	ND	9.0	ND	1.2
E_1	208	180.8	23.0	ND	5.6	ND	0.76
E_2	360	73	21.1	0.3	7.2	ND	0.92

ND = Not Detected.

The results also show a higher iron content for the tonic (Dubonnet and Ambassadeur) than Ozde bottled wines (Table 3.3). These results are, however, in agreement with those reported by Paul and Southgate (1978), but are generally lower when compared with those of Amerine and Ough (1969). It has been pointed out (Thouks, 1974) that low iron content could be, at least in part, due to the use of stainless steel equipment in wineries these days. Variation of one result from another may probably be as a result of differences in iron content of various soils. It may also be attributed to the differencein degree of fruit contamination during harvesting and transportation. With the exception of sample A_1 (Dark Lady), the entire result is within the 7 - 10mg/l range reported by Amerine and Ough (1980), and by this, it could be said that the wines are neither deficient in iron nor contain excess iron.

Zinc is present in all the samples, even though the result does not follow any definite pattern as seen in the case of iron (Table 3.3). The amount of copper is also found to vary irregularly, with some samples having copper content of below detectable level. These results are slightly higher than those reported by Amerine and Ough (1969, 1980) and Paul and Southgate (1978);

but lower than those of Nordi et al (1976). The amounts of zinc and copper are generally low enough and should not therefore, constitute health hazard and colour problem.

TABLE 3.4

METAL CONTAMINANTS (Pb and Cd) IN WINES (mg/l)

Sample	Cd	Pb
A_1	ND	8.0
A_2	ND	6.4
B_1	0.5	6.4
B_2	0.3	5.4
C_1	ND	11.6
C_2	ND	9.4
D_1	0.4	13.2
D_2	0.4	2.0
E_1	ND	ND
E_2	ND	4.0

ND = Not detected

Lead and Cadmium, the non-essential toxic trace metals are found to be quite high in the wines, almost higher than human tolerable limit, especially in the case of lead, where this has been set at about 0.2mg/l. Cadmium limit has not yet been

established, but FAO/WHO (Reilly, 1980) put a tolerable weekly intake of between 0.3 - 0.4 mg for an adult. The results (Table 3.4) also show that only the tonic wines (Ambassadeur and Dubonnet) contain cadmium within detectable range, with sample B_1 (Ambassadeur) having a maximum of 0.5 mg/l. Lead concentration in the wines, however, does not vary regularly, but the results indicate, with the exception of sample E_1 (White Lady) that all others contain lead far in excess of human tolerable limit. It could also be seen when compared with results of Amerine and Ough (1980), Modi et al (1976) and Nagy et al (1976) that lead concentrations far exceed those reported by these authors. No obvious reasons can be attributed to this variation, but it is said to be dependent, at least in part, on the location of the vineyard (Nagy, et al, 1976).

3.10 SUMMARY AND CONCLUSION.

It could be seen from the results of the analysis that, although the samples show some variations from results of previous works, it cannot be concluded that these wines are adulterated. These variations are expected, for they are brought about by variation in starting materials and method adopted in winemaking. Lower potassium, higher sodium and lead then results of previous works has been observed in most samples. Percent alcohol content, by volume, does not appear to comply with either the United States or United Kingdom System, with values almost equal to alcoholic strenght in degrees proof. It could probably mean that these systems are not adopted in this country, where the wines were bottled. The amount of tannins in the wines are also much lower than those reported by Amerine and Ough (1969, 1980) and other results of past analyses. Apart from the few important variations, the generality of the result compares favourably with those of previous analyses. Wines analysed could, therefore, be said to be free from adulteration, at least acute adulteration. It is, however, very essential that amount of lead in wines should be checked and possibly

reduced or eliminated completely. This, of course, can only be achieved by ensuring that vineyards are not located where the grapes can pick up toxic automobile emissions. Irrigating water, as well as containing vessels should also be free from contamination

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APPENDIX 1

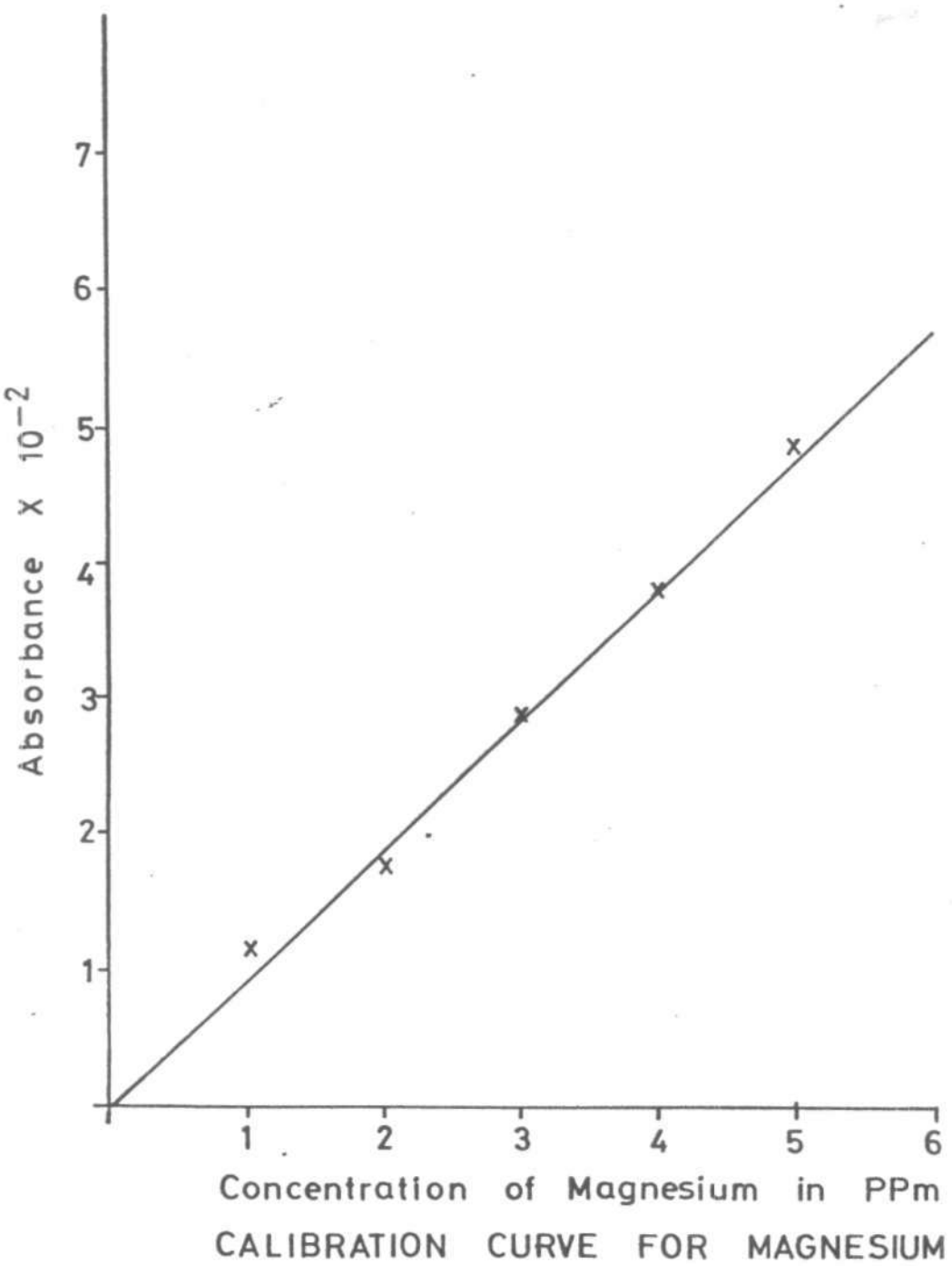
PREPARATION OF METAL STOCK, CALCIUM AND LANTHANUM SOLUTIONS

Metal/salt used (Metal detd)	Mass used, g	Method of preparation
Lead nitrate (Pb)	1.598	Dissolved in 200ml of deionised water, 1.5ml of concentrated HNO_3 added, and solution diluted to 1000ml.
Magnesium sulphate heptahydrate (Mg)	5.007	Dissolved in about 200ml of deionised water, 1.5ml of conc. HNO_3 added and solution diluted to 500ml, with deionised water.
Cupric sulphate pentahydrate (Cu)	3.929	Dissolved in 200ml of deionised water, 1.5ml conc. HNO_3 added and solution diluted to one litre with deionised water
Sodium chloride (Na)	2.542	Dried at 140°C and dissolved in a few millilitres of deionised water. 1.5ml of conc. HNO_3 added and solution made up to 1000ml with deionised water.
Manganous sulphate monohydrate (Mn)	3.076	Dissolved in about 200ml of deionised water. 1.5ml of conc. HNO_3 added and solution made up to one litre with deionised water

Appendix 1 (cont'd)

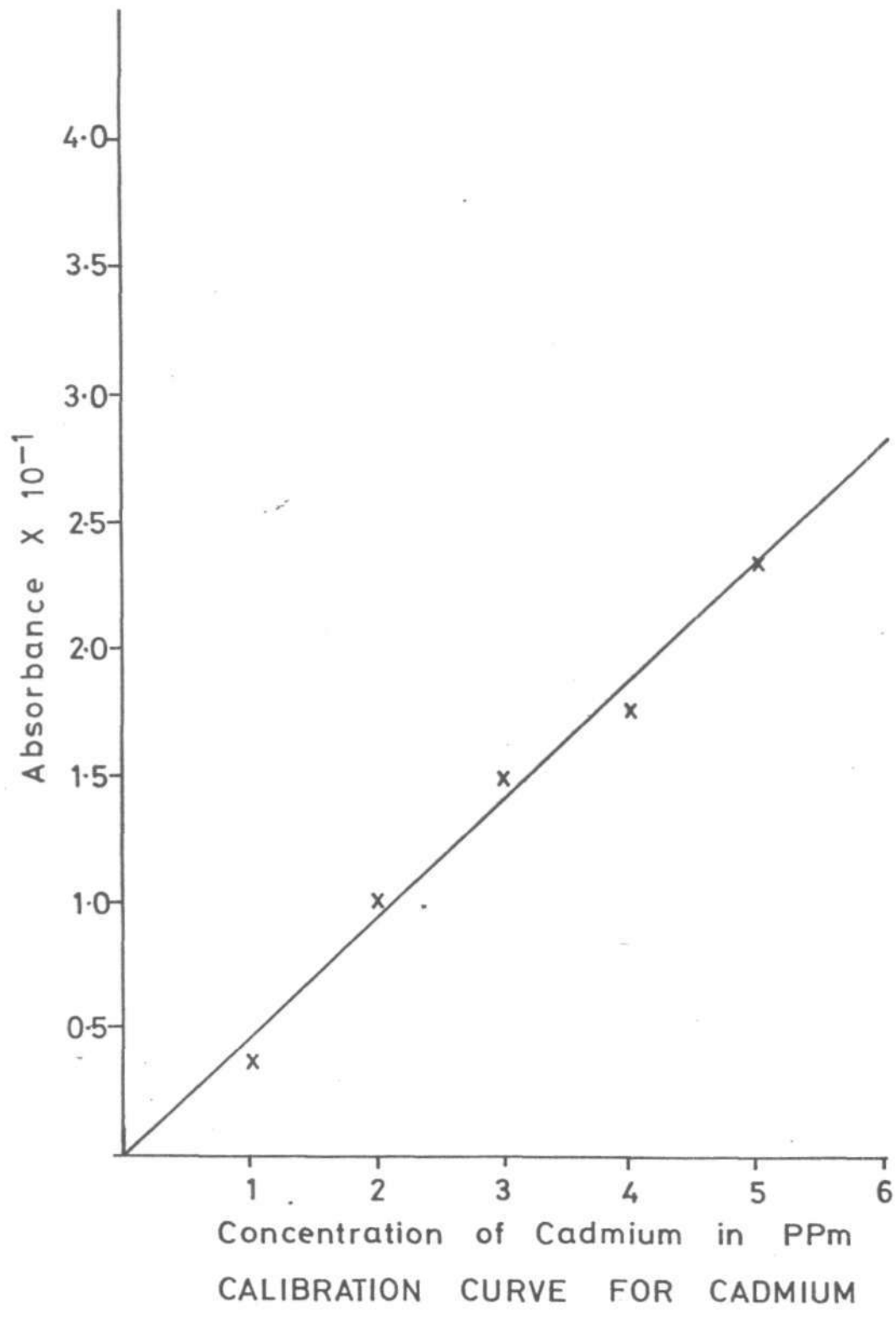
Metal/salt used (metal detd)	Mass used, g	Method of preparation
Potassium chloride (K)	1.907	Dried at 110°C, dissolved in about 200ml of deionised water. 1.5ml of conc. HNO_3 added and solution diluted to 1000 ml.
Ammonium ferrous sulphate (Fe)	3.511	As for Mn
Cadmium nitrate (Cd)	2.103	Dissolved in about 200ml of deionised water. 1.5ml of conc. HNO_3 added and solution diluted to 1000ml.
Zinc metal (Zn)	1.000	Dissolved in 20ml of 1:1 HNO_3 and diluted to one litre with deionised water.
Lanthanum solution	29.325	Dissolved in about 125ml of concentrated HCl and diluted to 500ml with deionised water. Mixed thoroughly with the samples while determing magnesium.
Calcium solution	0.630	Dissolved in 10ml of conc. HCl 200ml of deionised water added and slightly heated. Cold solution diluted to 1000ml. Mixed with samples while determining Fe and Mn.

Appendix 2a



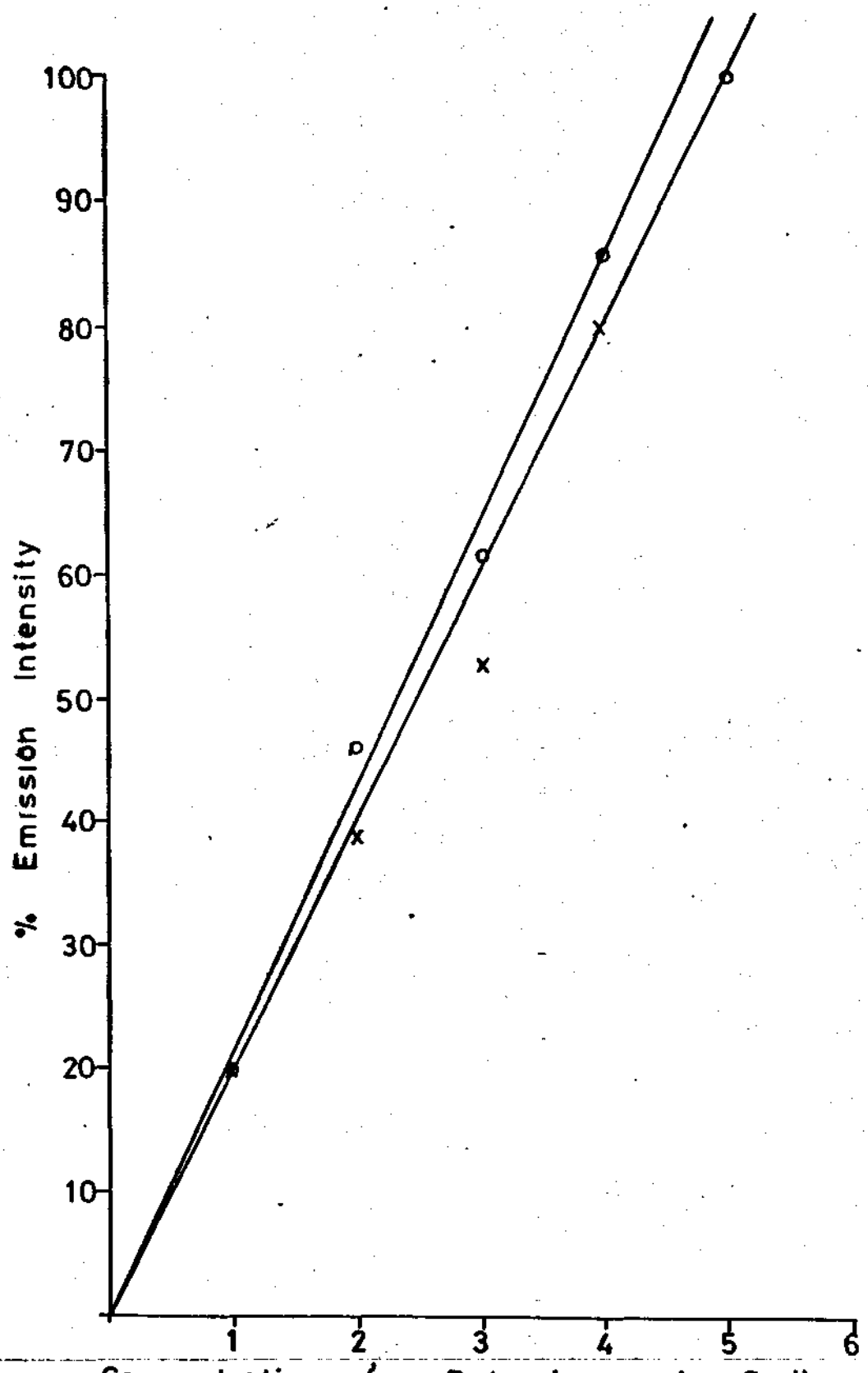
CALIBRATION CURVE FOR MAGNESIUM

Appendix 2b



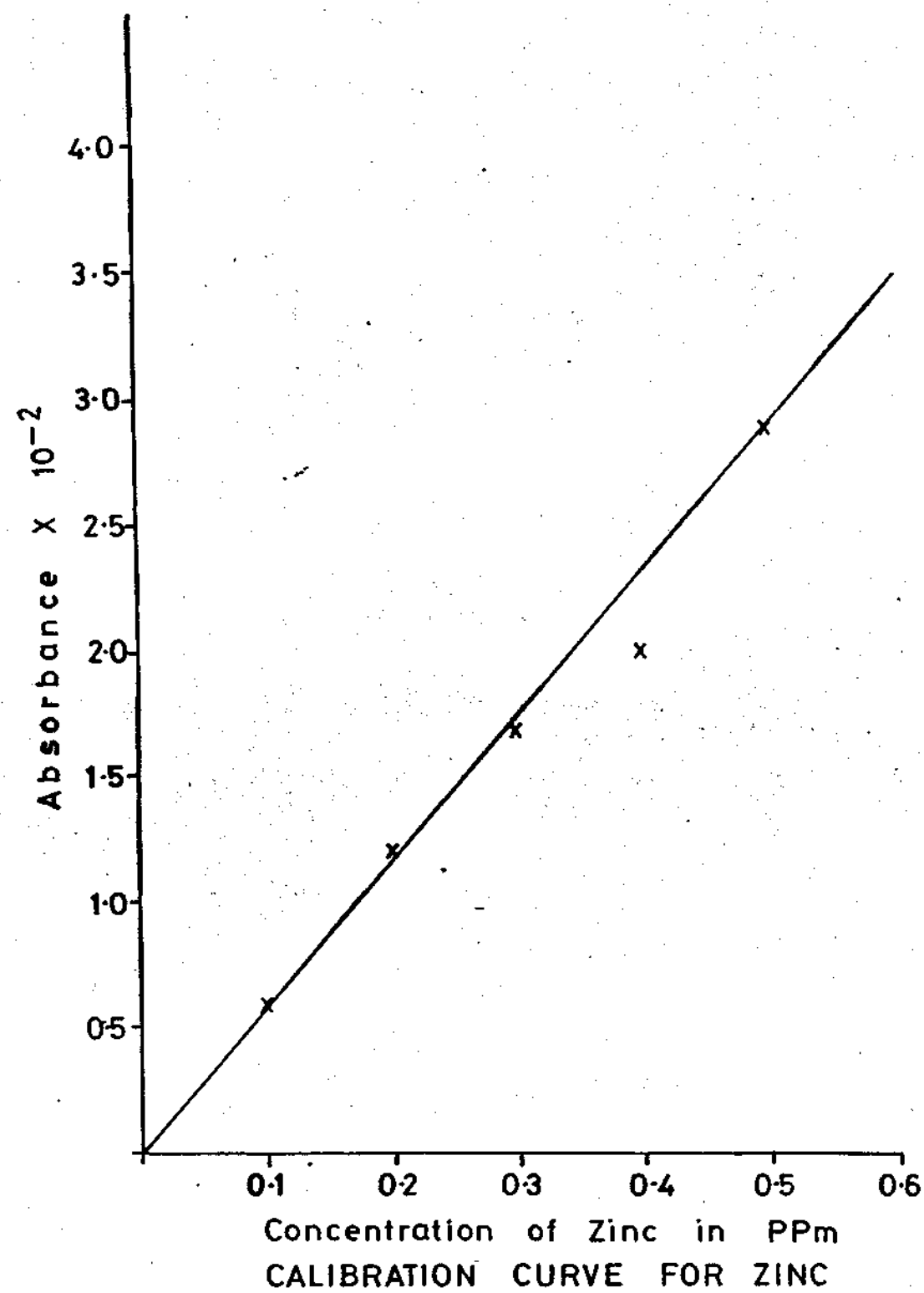
CALIBRATION CURVE FOR CADMIUM

Appendix 2c



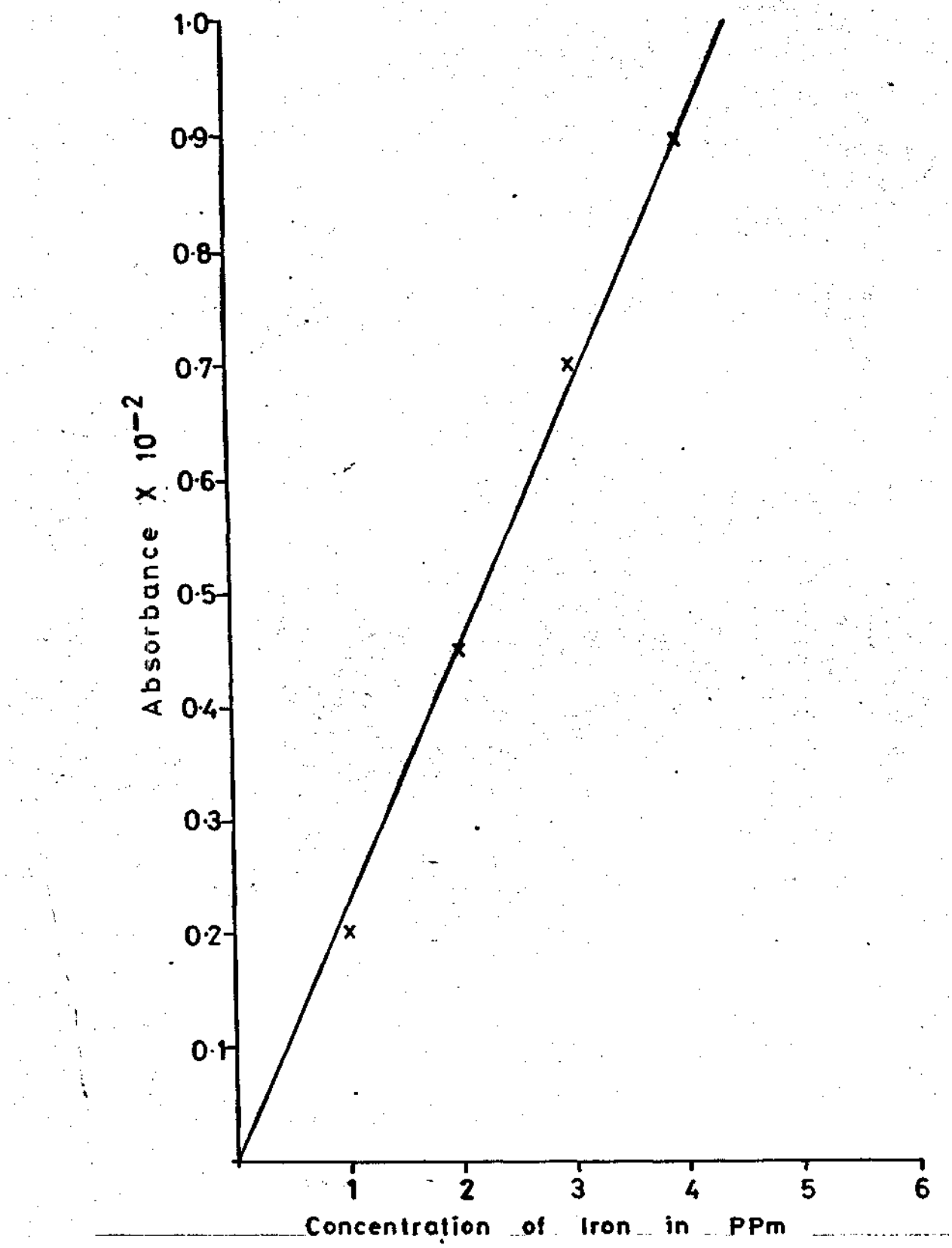
CALIBRATION CURVES FOR K AND Na

Appendix 2d



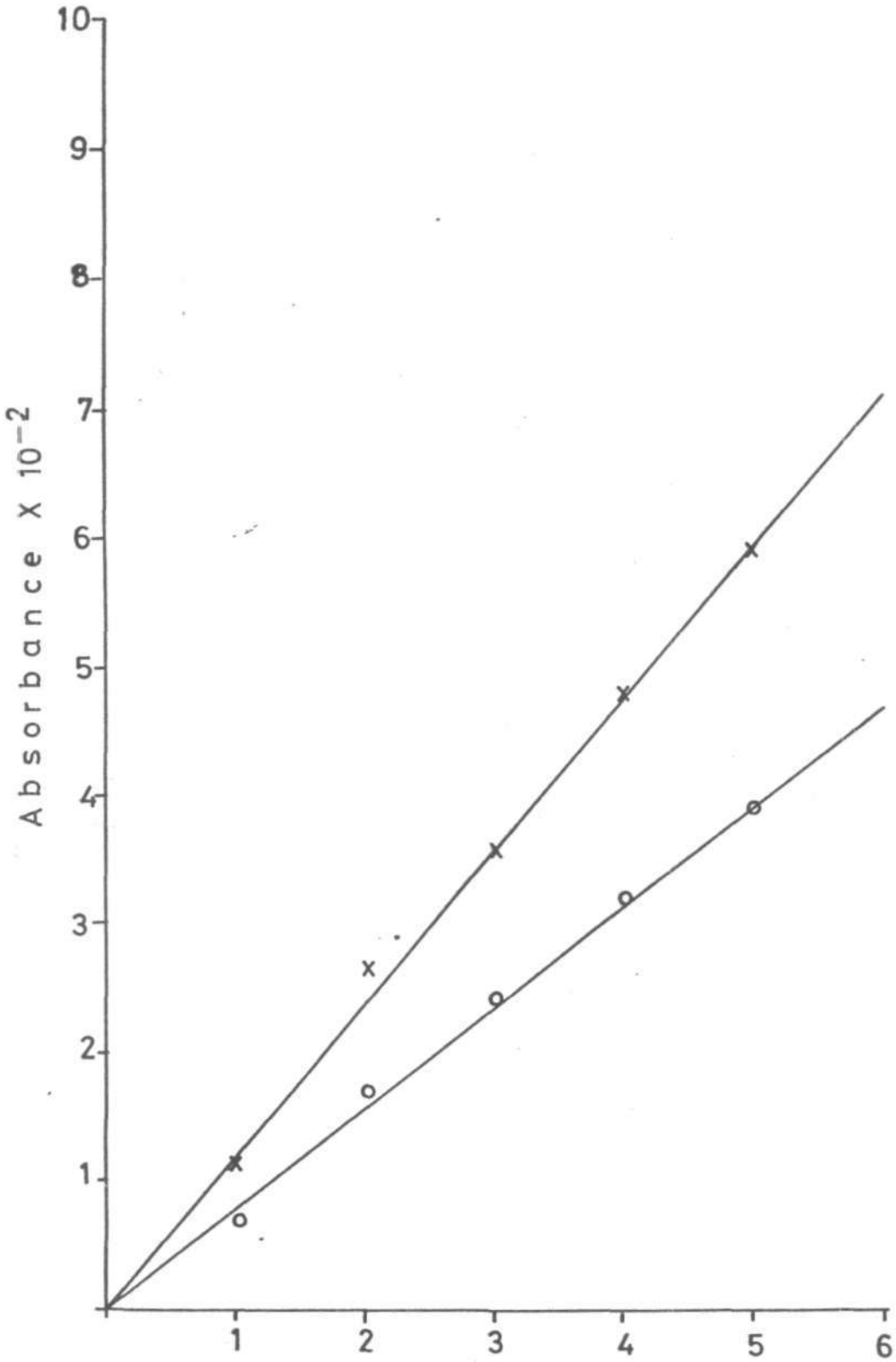
CALIBRATION CURVE FOR ZINC

Appendix 2e



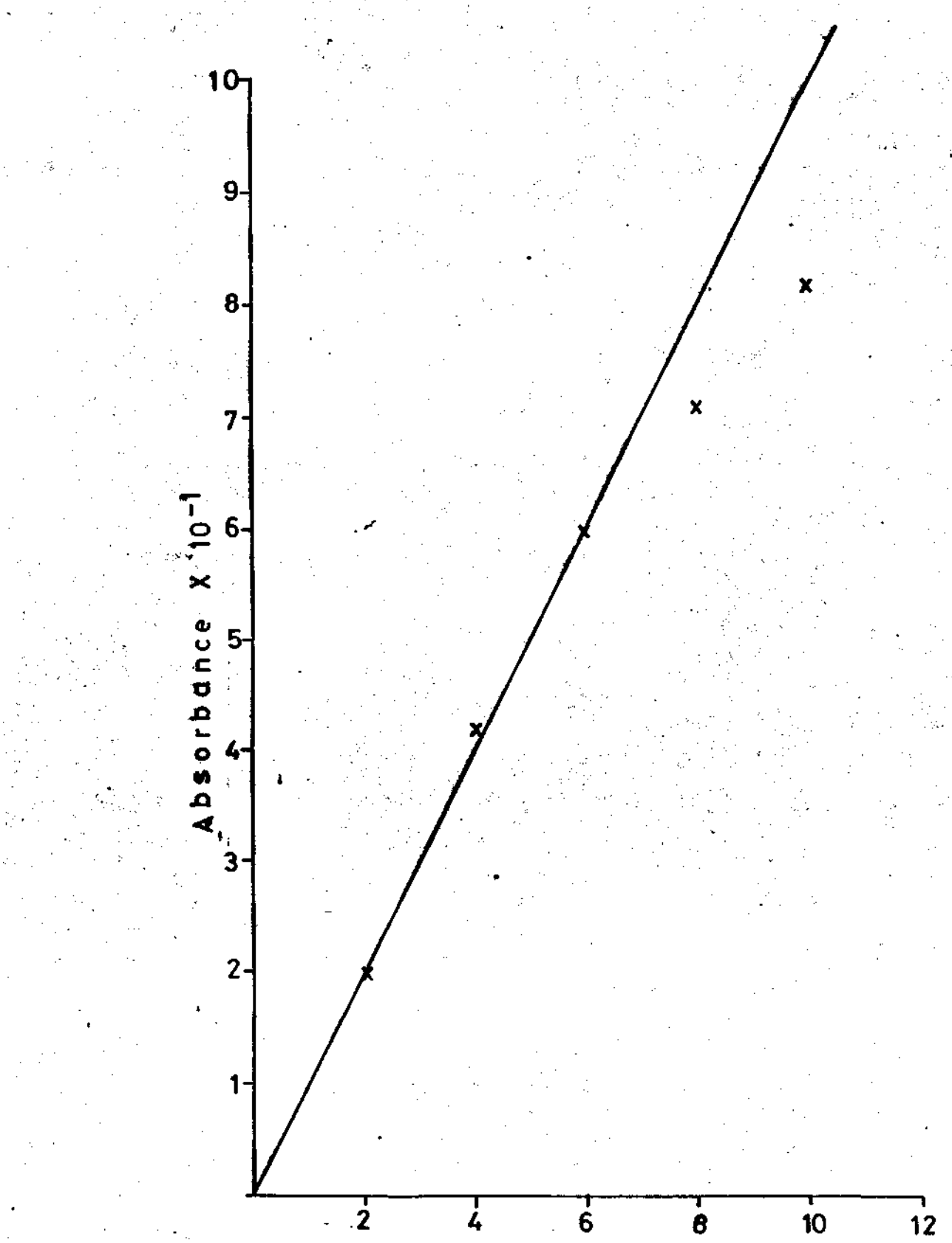
CALIBRATION CURVE FOR IRON

Appendix 2f



CALIBRATION CURVES FOR COPPER AND LEAD

Appendix 2g



CALIBRATION CURVE FOR TANNINS

APPENDIX 3a

Percent Alcohol, Degrees proof for United Kingdom and United States of American From Tayleur (1973) pages 304 - 305.

Alcohol % by volume	Degrees proof U.K.	Degrees proof U.S.A
2	3½	4
4	7	8
6	10½	12
8	14	16
10	17½	20
12	21	24
14	24½	28
16	28	32
18	31½	36
20	35	40
22	38½	44
24	42	48
26	45½	52
28	49	56
30	52½	60
32	56	64
34	59½	68
36	63	72
38	66½	76
40	70	80
42	73½	84
44	77	88
46	80½	92
48	84	96
50	87½	100
52	91	104
54	94½	108
56	98	112
57.14	100	114.28
58	101½	116

Appendix 3a (cont'd)

Alcohol % by volume	Degrees proof U.K.	Degrees proof U.S.A.
60	105	120
62	108½	124
64	112	128
66	115½	132
68	119	136
70	122½	140
72	126	144
74	129½	148
76	133	152
78	136½	156
80	140	160

APPENDIX 3b

Specific gravity and Extract concentration at 20°C.
From Amerine and Ough (1980): page 30.

Specific gravity	Extract (g/100ml)	Specific gravity	Extract (g/100ml)
1.000	0.00	1.031	8.03
1.001	0.26	1.032	8.29
1.002	0.51	1.033	8.55
1.003	0.77	1.034	8.81
1.004	1.03	1.035	9.07
1.005	1.29	1.036	9.33
1.006	1.54	1.037	9.59
1.007	1.80	1.038	9.85
1.008	2.06	1.039	10.11
1.009	2.32	1.040	10.37
1,010	2.58	1.041	10.63
1.011	3.10	1.042	10.90
1.012	3.36	1.043	11.16
1.013	3.62	1.044	11.42
1.014	3.88	1.045	11.68
1.015	4.13	1.046	11.94
1.016	4.39	1.047	12.20
1.017	4.65	1.048	12.46
1.018	4.91	1.049	12.72
1,019	5.17	1.050	12.98
1,021	5.43	1.051	13.24
1.022	5.69	1.052	13.50
1,023	5.95	1.053	13.76
1.024	6.21	1.054	14.03
1.025	6.47	1.055	14.29
1.026	6.73	1.056	14.55
1.027	6.99	1.067	14.81
1.028	7.25	1.058	15.07
1.029	7.51	1.059	15.33
1.030	7.77	1.060	15.59

Appendix 3b (cont'd)

Specific gravity	Extract (g/100ml)	Specific gravity	Extract (g/100ml)
1.061	15.89	1.095	24.78
1.062	16.12	1.096	25.04
1.063	16.38	1.097	25.31
1.064	16.64	1.098	25.57
1.065	16.90	1.099	25.84
1.066	17.16	1.100	26.10
1.067	17.43	1.101	26.36
1.068	17.69	1.102	26.63
1.069	17.95	1.103	26.89
1.070	18.21	1.104	27.15
1.071	18.48	1.105	27.42
1.072	18.74	1.106	27.68
1.073	19.00	1.107	27.95
1.074	19.26	1.108	28.21
1.075	19.52	1.109	28.48
1.076	19.78	1.110	29.74
1.077	20.05	1.111	29.00
1.078	20.31	4.112	29.27
1.079	20.58	1.113	29.53
1.080	20.84	1.114	29.80
1.081	21.10	1.115	30.06
1.082	21.36	1.116	30.33
1.083	21.62	1.117	30.59
1.084	21.89	1.118	30.86
1.085	22.15	1.119	31.12
1.086	22.41		
1.087	22.68		
1.088	22.94		
1.089	23.20		
1.090	23.47		
1.091	23.73		
1.092	23.99		
1.093	24.25		
1.094	24.52		

APPENDIX 3c

Proof, percent Ethanol by volume, weight and specific gravity, at 20°C. From Amerine and Ough (1980), page 28 - 29.

Proof	Alcohol (wt.%)	Alcohol (wt. %)	Specific gravity
0.0	0.00	0.00	1.00000
1.0	0.50	0.40	0.99925
2.0	1.00	0.795	0.99851
3.0	1.50	1.19	0.99777
4.0	2.00	1.593	0.99704
5.0	2.50	1.99	0.99633
6.0	3.00	2.392	0.99560
7.0	3.50	2.80	0.99490
8.0	4.00	3.194	0.99419
9.0	4.50	3.60	0.99350
10.0	5.00	3.998	0.99281
11.0	5.50	4.40	0.99214
12.0	6.00	4.804	0.99149
13.0	6.50	5.210	0.99084
14.0	7.00	5.612	0.99020
15.0	7.50	6.02	0.98956
16.0	8.00	6.422	0.98894
17.0	8.50	6.83	0.98832
18.0	9.00	7.234	0.98771
19.0	9.50	7.64	0.98711
20.0	10.00	8.047	0.98650
21.0	10.50	8.45	0.98590
22.0	11.00	8.862	0.98530
23.0	11.50	9.27	0.98471
24.0	12.00	9.679	0.98412
25.0	12.50	10.80	0.98354
26.0	13.00	10.497	0.98297
27.0	13.50	10.90	0.98239
28.0	14.00	11.317	0.98182

Appendix 3c (cont'd)

Proof	Alcohol (wt. %)	Alcohol (wt. %)	Specific gravity
29.0	14.50	11.72	0.98127
30.0	15.00	12.138	0.98071
31.0	15.50	12.54	0.98015
32.0	16.00	12.961	0.97960
33.0	16.50	13.37	0.97904
34.0	17.00	13.786	0.97850
35.0	17.50	14.19	0.97797
36.0	18.00	14.612	0.97743
37.0	18.50	15.02	0.97690
38.0	19.00	15.440	0.97638
39.0	19.50	15.84	0.97585
40.0	20.00	16.269	0.97532
41.0	20.50	16.67	0.97479
42.0	21.00	17.100	0.97425
43.0	21.50	17.51	0.97372
44.0	22.00	17.933	0.97318
45.0	22.50	18.34	0.97262