EFFECTS OF PROCESSING CONDITIONS AND STORAGE ON HONEY QUALITY

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my late father Mr. Kasolia Mutangili and my mother Veronica Nguna who gave the first 'Go'. It is also a dedication to my wife Beatrice Nthenya and my children Kyuli, Nguna and Mutiso for their love and encouragement throughout this study period.

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Table of contents

Table of Contents Cont'd

Table of Contents Cont'd

Table of Contents Cont'd

Table of Contents Cont'd

List of Tables

List of Tables Cont'd

List of Tables Cont'd

List of Figures

List of Plates

ABSTRACT

Fresh Kenya crude honey was collected from two districts and analyzed for their physico-chemical properties before and after heat processing at each of the temperatures for 20, 25 and 30 minutes at 45°, 50°, 55° and 60°C. The physico-chemical properties analyzed included residual diastase (amylase) activity, hydroxmethylfurfural (HMF) content, colour and rate of crystallization. Processed honeys were also analyzed for the same during six months of storage room temperature (18±4°C), 22°, 28° and 35°C.

Significant differences at 5% level were found in crystallization rate between all processing temperatures used. Levels of HMF differed significantly (P=0.05) between unheated and heated samples and also between samples heated at 45°C and those processed at other temperatures. Samples heated at 50°C also differed significantly (P=0.05) in HMF content with those heated at 55° and 60°C. Generally, increased temperatures resulted in increased HMF production. The colour as measured in terms of optical density at 440 nm showed significant differences (p=0.05) between unheated and heated samples, while heated sampled did not differ significantly among themselves. However, there was a positive correlation between increased processing temperature and increased optical density in all samples studied. No significant differences (P=0.05) in diastase activity were found among all

honey samples studied, although there were noticeable decreases in diastase residual activity with increased processing temperatures.

Increase in storage temperature and/or time resulted in increased rates of HMF production with high temperatures (28°C and 35°C) showing HMF values above those recommended for table honey (40 ppm) after six months of storage. The rate of diastase inactivation increased with increased storage temperature and/or time. Within four months' storage at 35°C, the residual diastase activity had been lowered to below number of 8, the minimum recommended for table honey. The optical density (for colour) increased with increased storage time and/or temperature. High storage temperatures (28° and 35°C) showed significantly higher (P=0.05) optical density and visual colour than those at lower temperatures (22° and 18 $\pm$ 4°C . Increased storage temperature and/or time resulted in decreased rate of crystallization with most samples stored at 28° and 35°C showing no crystallization at all after six months of storage.

Processing at 55°C for 25-30 minutes and storage at 25-26°C could be recommended as the most appropriate for honey processing and storage.

1. INTRODUCTION

Beekeeping has been practised in Kenya since time immemorial. Although in the past, honey was produced using traditional log hives, present production uses both the traditional hives and the modern Kenya Top Bar (KTB) hives. Honey from the traditional hives forms the bulk of traded honey in Kenya (Kasolia, 1983).

Honey is normally harvested and mashed, the process during which foreign matter which includes dead bees and their parts, brood (bee larvae), pollen, wax particles, pieces/particles of charcoal (from the flames used for harvesting), are incorporated. This extraneous matter apart from impairing the natural honey flavour and appearance also hastens honey crystalization during storage. Although crystallization is not a loss of quality, many consumers liken it to adulteration with sugars from other sources. Today's retail market favours processed honey which remains liquid and has an attractive colour and appearance.

Of the total honey production, about 70% is used unprocessed (crude) for preparation of traditional fermented beverages, while the remaining 30% is processed for table and/or industrial uses. The latter market segment is, however, growing, while the former seems to be declining (Gatere et al., 1985).

Processing of honey in Kenya is carried out in both large and small refineries. The large refineries are owned by either producer cooperatives or private firms, while the small scale refineries are chiefly owned by individual packers. The processing temperatures and times vary among processors but usually range from between 38° to 80°C, and five minutes to several hours respectively. This results in processed honeys with varying chemical composition, colour, flavour and propensity to crystallize, and therefore different shelf lives. Changes are in turn dependent on temperature of storage.

Most processed honeys found on the shelves in retail stores in Kenya are of quality below the minimum specified by Kenya Standards for honey. This can be attributed to overheating during processing and poor storage conditions. The honeys also do not meet the export requirements for most countries (Kenya is a net exporter of honey). There is therefore a need to investigate on the proper processing time and temperature conditions that result in honey of acceptable market quality and maximum shelf-life under given storage temperatures. The dependence of honey quality deterioration on the storage conditions in Kenya have also not been investigated. The aim of the present project was therefore to determine most appropriate processing and storage conditions for Kenyan honey under the following objectives:

1. To determine the processing temperature and holding time necessary to facilitate straining, while at the same time giving a finished product of high quality.

2. To investigate the rate of crystallization of strained honey processed at different temperatures and for different times during storage at four different temperatures.

3. To determine the keeping quality of processed honey at different storage temperatures.

2.0 LITERATURE REVIEW

2.1. BIOLOGICAL ORIGIN OF HONEY

Honey is defined as "the sweet substance produced by honeybee from the nectar of blossoms or from secretions of or/on living parts of plant which the bees collect, transform and combine with other substances, ripen then store in honey combs" (Codex Alimentarius Commission, 1969; Kenya Standards for honey; 1985). Behind this definition is therefore a whole world of honeybee activity, because consideration is not only given to the finished product but also to the raw material, source of raw material and its conversion to honey. The material collected by bees are mainly unprocessed sugar-containing plant fluids and honeydew (the secretion of plant sucking insects). Honey is therefore a product of the honeybee colony activity, not of the plant (Maurizio, 1962; Petrov, 1974).

According to Maurizio (1962), the raw material for honey in the temperate zones is primarily nectar from flowers of the higher plants, as well as honeydew, while in the subtropical and tropical climates honey is mainly derived from other sources, such as nectar from extrafloral nectaries or other sweet plant fluids.

Both honey-nectar and honeydew originate from the fluid or sap circulating through the phloem of higher plants. Nectar is produced by the plant in the tissue of nectaries, while honeydew is a secondary metabolite of the plant-sucking insects such as

aphids. The bee collects these materials and processes both of them into honey in the same manner. The product from nectar is referred to as blossom or extrafloral honey, while that from honeydew is honeydew honey (Codex Alimentarius, 1969). The honey found in some regions is usually a mixture of the two types.

2.1.1. The Phloem Sap

Phloem sap contains about 20% sugars and about 0.9% nitrogenous compounds . However, the composition of the phloem sap is by no means standard and varies between plant species. Phloem sap from some plant species such as Robinia psedoacacia, Acer plantanoides, consists of sucrose only, while that from plants such as Ailantus altissima, fructose, glucose, stachiose, raffinose and verboscose also occur. In addition to the variation between plant species, the pattern of sugars in the phloem sap also shows seasonal changes. (Zimmermann, 1957; Parker, 1958; Ziegler et al., 1959).

2.1.2. Nectar

The composition of nectar is different from that of phloem sap (Ziegler, 1956, Luthge, 1961) 1956). Nectar is essentially an aqueous solution of sugars whose composition varies with plant species. The concentration of sugars in nectar is generally higher than that in phloem sap. The total sugar content in nectar varies from 3% to 80% of the total weight and 90-95% of the total solid matter, depending on the flower species, weather, climate, time of day or year, moisture in the air and soil

(Vansell et al; 1942; Schuell, 1952, Crane, 1980). Bees prefer nectar with a high sugar contents and usually do not gather those with less than 15% sugar (Wykes, 1952).

Nectar can be divided into three groups according to sugar constitution. The first group is derived from phloem sap of the plant (Rhododendron ferruqineum) and contains mainly sucrose. The second group of nectars contains sucrose, glucose and fructose in roughly equal amounts (Melitotus alba), while the third group of nectars contains glucose and fructose with hardly any sucrose (Taraxacum officinale, Castanea sativa), but more fructose than glucose (Furgala et al.,1958; Maurizio, 1959; Crane, 1980). Freshly collected nectar from flowers usually contains the three sugars, Sucrose, fructose and glucose, with maltose and some higher polymeric sugars also appearing in nectars from some plants and in nectars that have been stored for sometime. The high polymeric sugars come about due to the action of enzymes secreted by the nectary. These enzymes are also capable of breaking down the sugar molecules. The enzymes are secreted with the nectar, and their action continues in the secreted nectar and in the honey (Maurizio, 1962). According to Maurizio (1962), hydrolysis of the complex sugars to simpler sugars is accompanied by synthesis of new sugars of high molecular weights, by group transference. This reaction can proceed through breakdown of sucrose in transfructosidation or in transglucosidation. A simplified process of transfrucosidation is as follows:

Sucrose + Enzyme ---> glucose + fructose - enzyme complex

The fructose - enzyme complex is then transferred to a so called 'acceptor' sucrose forming a new trisaccharide:

Fructose - enzyme + sucrose (acceptor)----> glucose - fructose - fructose + enzyme

Tetrasaccharides and oligosaccharides are formed similarly. This mechanism leads to accumulation of glucose in the system. In this process, the fructose residue can be transferred to other sugars, producing fructose-containing oligosaccharides of these sugars. Transglucosidation will on the other hand give glucose-containing oligosaccharides and a corresponding increase in fructose concentration of the substratum (Bacon et al., 1950; Bacon, 1952; White, 1952). The oligosaccharide formed and the ratio of fructose to glucose depend on the type of sucrose hydrolysis. These processes take place similarly in both honeydew and ripe honey. In addition to sugars, nectars also contain small amounts of other substances such as nitrogenous and phosphorous compounds, organic acids, minerals, vitamins, enzymes, essential oils and other substances which contribute to the flavour (Beutler, 1953).

2.1.3 Conversion of nectar to honey

The foraging bees draw up nectar or honeydew with their long mouth parts into their honey sacs which are specialised organs along the alimentary canal. These sacs are sealed off from the rest of the digestive tract by the proventricular valve (Maurizio, 1962). The bees usually return to the hive when their

sacs are full. In the sacs, the nectar is diluted with saliva which contains secretions from several glands, mostly the hypopharyngeal glands in the bees head, which provide the enzymes invertase, diastase and glucose oxidase used in elaborating honey (Maurizio, 1962; Crane, 1980). On reaching the hive, the forage bees pass their load to the house bees which undertake the task of honey ripening. In the undercrowded parts of the hive the house bees regurgitate from their honey sacs drops of the raw materials spreading them out into thin films with their retroverted proboscis. After about 5 - 10 seconds, they sack the drops back into the mouth. This process is repeated for about 15 - 20 minutes, during which the raw material is further mixed with glandular secretions and at the same time exposed to the warm dry atmosphere of the hive, thus losing some moisture. In this way the raw material containing initially 25 - 40% dry matter is converted into half-ripe honey of about 60 - 65 dry matter. Upon completion of the foregoing part of the ripening process, the house bees deposit the half-ripe honey into the cells of the comb where subsequent ripening takes place through evaporation of water into the stream of dry air flowing over the honey through the ventilation system of the hive (Park, 1933; Reinkardt, 1939). When ripening is complete, the honey contains no more than 20% moisture. Then the bees cover the honey cells with beeswax (capping), which prevents absorption of water by the hygroscopic honey thereby minimizing the risk of fermentation of the honey in the hive (Maurizio, 1962; Petrov, 1974; Crane, 1980).

2.2 HONEY PRODUCTION IN THE WORLD

Honey is produced in almost every country of the world. It was one of man's earliest foods and it has been valued throughout successive civilisations. Today, the popularity of honey is increasing as never before. Table 1 and 2 show the World's honey production and trade respectively between the period 1986 - 1988.

Most of the world's honey is produced from one species of honeybee, the Apis merrifera. In Asia, however, some honey is produced from other bee species such as Apis cerana, Apis florea, Apis dorsatas, but it is unlikely that these sources account for more than 10% of the world's honey crop. About 2 % is also perhaps produced by non-Apis bees such as Apini, Meliponini and Bombini, all of them tropical species (Crane, 1975).

Table 1. World Honey Production (1986-1988) in Metric Tons

	1986	1987	1988
Africa	98176	102280	104543
North America	200166	213498	197841
South America	66078	70339	72488
Asia	300771	326901	324856
Europe	188377	170377	178187
U.S.S.R	205000	190000	192000
Oceania	36839	170377	178187
Total	1095406	1109442	1110385

Source: FAO Production yearbook Vol. 42, 1988

Table 2. World Honey Trade (1986 - 1988) in Metric Tons

	Imports			Exports		
	1986	1987	1988	1986	1987	1988
Africa	192	162	126	192	255	236
North America	54208	27352	26499	89296	69161	70831
South America	599	1266	1015	37644	40773	49759
Asia	44749	49542	48428	86835	72486	53154
Europe	186244	184971	180165	57309	53698	54746
U.S.S.R	210	506	513	20607	20871	17438
Oceania	156	183	214	17809	13427	15631
Total	286358	263982	256960	309692	270671	261795

Source: FAO Trade yearbook Vol. 42, 1988

2.2.1. Honey Production in Kenya

Honey production in Kenya is carried out by small scale farmers who are scattered all over the country, where floristic influence to honeybee is possible. Most beekeeping activities are concentrated in the arid and semi-arid regions of the country, mainly in Eastern, Coast, and Rift Valley provinces of the country. These are the areas where traditional beekeeping has been practised since time immemorial.

There are many bee plants in Kenya (Appendix 1). Most of the honey in the country is polyfloral with Acacia Spp as the predominant source (Kahenya et al., 1985).

In 1988, Kenya's honey production was estimated at 15000 metric tons. Of the total production, about 90% came from traditional log hives, then estimated at 2 million in number, while the remaining 10% came from improved Kenya Top Bar hives (MLD, 1988).

About 70% of Kenya's honey is used in unprocessed (crude) form for production of traditional beers, with the remaining percentage (30%) being processed for table and industrial uses. For table consumption, the honey is used as a spread on bread, as a sweetener in place of sugar, for preparation of fruit beverages and for home baking. Industrially, honey in Kenya is used in confectionary, pharmaceutical, tobacco and baby food manufacturing industries (Gatere et al., 1985). Some processed honey is also exported as is shown in Table 3. The Table also shows that while both exports and imports have been variable, domestic production has increased steadily over the period 1981 - 1988.

Table 3. Kenya Honey Production, Exports and Imports 1981 - 1988 in Metric Tons.

Year	Production:	Export:	Import:
1981	11120	3.1	13.5
1982	11400	11.9	-
1983	12800	16.5	1.4
1984	13000	1.0	2.3
1985	14000	2.3	1.0
1986	15000	8.7	2.3
1987	15200	11.9	-
1988	15400	1.0	1.0

Source: Annual reports, Beekeeping Branch, Ministry of Live stock Development - Kenya.

2.3. COMPOSITION OF HONEY

Honey has variable composition depending on plant source, climate, environmental conditions and beekeepers skills. So far 181 different substances have been identified in honey, some not known to exist elsewhere (Crane, 1980). Table 4 shows average and extreme percentages of the major components of honey from the United States of America.

Table 4. Average Characteristics of Honey.

Components	Average	Standard deviation	Range
Moisture,(%)	17.2	1.46	13.4 - 22.9
Fructose (Levulose) %	38.19	2.07	27.25 - 44.26
Glucose, (dextrose)	31.28	3.03	22.03 - 40.75
Sucrose, (%)	1.31	0.95	0.25 - 7.57
Maltose, (%)	7.31	2.09	2.74 - 15.98
Higher Sugars, (%)	1.50	1.03	0.13 - 8.49
Undetermined, (%)	3.1	1.97	0.00 - 13.2
pH	3.91	-	3.42 - 6.10
Free acid, meq/kg	22.03	8.22	6.75 - 47.19
Lactose, meq/kg	7.11	3.52	0.00 - 18.76
Total acid, Meq/kg	29.12	10.33	8.68 - 59.49
Lactone, free acid	0.335	0.135	0.00 - .950
Ash, (%)	0.169	0.15	0.02 - 1.028
Nitrogen, (%)	0.041	0.026	0.00 - 1.33
Diastase number	20.8	9.76	2.1 - 61.2

Source: White et al. (1962).

2.3.1. Moisture Content of Honey

The moisture content of honey is important as regards its keeping quality. Variation in water content occurs as a result of differences in atmospheric humidity before and after honey is harvested. The principal short-term instabilities of honey are crystallisation and fermentation, liability of both of which are related to moisture content. The tendency to crystallize has been associated with the glucose/water ratio (White et al., 1962; Hadorn et al., 1974). Fermentation mainly by osmophilic yeasts will occur if the combination of moisture content, temperature and yeast count is favourable (Lochhead, 1933). Table 5 shows the dependence of the ease of honey to ferment on the moisture content and yeast count.

Table 5. Dependence of Moisture Content and Yeast Count on Liability of Honey Fermentation

Moisture Content %	Liability
Below 17.1	None
17.1 - 18.0	None if yeast count <1000/gm
18.1 - 19.0	None if yeast count <10/gm
19.1 - 20.0	None if yeast count <1/gm
Above 20.0	Always liable

Data of Lochhead (1933) based on 319 honey samples.

2.3.2 Carbohydrate Content of Honey

Carbohydrates form 70-80% of total honey constituents (Petrov, 1974). The sugars are responsible for much of the physical characteristic of honey, its crystallisation, hygroscopicity viscosity properties, energy values and so on (White, 1974). Fructose and glucose invariably account for 85 - 95% of the total honey sugars (Petrov, 1974; White, 1975). Apart from monosaccharides, disaccharides and higher sugars (Oligosaccharides) have been isolated and identified in honey. Research in the United states, Japan and Canada has identified at least eleven disaccharides in honey in addition to sucrose. Most of these sugars are quite rare and their isolation from honey was the first recovery from natural materials (White, 1975). The amount of reducing disaccharides (e.g. Maltose) in honey seems to increase during storage depending on the storage conditions. This has been attributed to enzyme activity.

One honey sample which had been stored for 36 years was reported to contain 16% maltose and related compounds (Crane, 1980). Siddiqui *et al*. (1968a) have reported the isolation and identification of at least eleven oligosaccharides. Table 6 gives a list of sugars in honey.

Table 6. Sugars so far found in Honey

Mono-saccharides	Disa-ccharides	Tri-saccharides	Oligo-saccharides
Glucose	Sucrose	Erlose	Isomaltote-traose
Fructose	Maltose	Theanderose	Isoma - Itopentaose
	Isomaltose	Panose	
	Maltulose	Maltotriose	
	Isomaltulose	1-Kestose	
	Nigerose	Isomaltotriose	
	Turanose	Melizitose	
	Kojibiose	Isopanose	
	Neotrehalose	Centose	
	Gentiobiose	3-a-Isomaltosyl-glucose	
	Laminaribiose	Raffinose	
	Leucrose	6^G-a-glucosyl-sucrose	
		Arabogalactomannan dextrantriose	

Source: Siddiqui and Furgala (1967, 1968) and Crane (1980)

2.3.4. Acidity of Honey

The flavour characteristic of honey is partly contributed by its acidity. The pH of honey averages 3.9 as has already been shown in Table 1. This level of acidity probably contributes to its stability against micro-organisms (White, 1975).

According to Stinson et al. (1960), gluconic acid in equilibrium with gluconolactone is the major acid in honey. It is produced by the action of the glucose oxidase normally present in honey on glucose (White et al., 1963). The reaction is very slow in full-density honey but fast in diluted honey. The combined effect of acidity and the hydrogen peroxide concurrently produced is thought to preserve nectar from spoilage during the ripening stage (White, 1978). Other organic acids that have been identified in honey include acetic, butyric, lactic and pyroglutamic (Stinson et al., 1960), citric and succinic (Nelson, et al., 1931), formic (Vogel, 1882, cited by Farnsteiner, 1908), maleic (Goldschmidt et al., 1955), Malic (Hilger, 1904), and oxalic (von Philips-born, 1952). The sources of many of these acids in honey are not yet known.

2.3.5. Mineral constituents

Honey varies widely in its mineral content. Table 4 shows an average ash content of 0.169 with a range of 0.020 - 1.028 for U.S. honeys. Potassium is the principal mineral constituting an average of about one-third of total ash, while sodium constitutes roughly one-tenth of the total mineral content (White et al.,

1962). Other minerals include iron, copper, calcium, manganese, magnesium, phosphorus, silicon, aluminium, chronium, nickel and cobalt. The mineral components of honey come through from the nectar unchanged. Dark honeys have a higher ash contents therefore higher mineral contents than light honeys (Luttge, 1962).

2.3.6 Enzymes in Honey

All natural honeys contain enzymes. The enzymes are some of the most interesting constituents of honey that have received greatest level of research over the years. The three most important enzymes in honey are diastase (amylase), invertase and glucose oxidase. Other enzymes are catalase and acid phosphatase (White, 1978; Crane, 1980).

2.3.6.1 Diastase (amylase)

Diastase or amylases are the starch digesting enzymes and have been shown to be present in honey. Because of their heat sensitivity they have been used considerably in honey quality control to test for severity of heat processing. Honey has both alpha and beta amylase activity. According to Lampitt et al., (1929), the optimal pH for the alpha- amylases is about 5.0 at 22 - 30°C temperature range and 5.3 at temperatures between 30 - 45°C. An optimal pH value of 5.3 has been reported for beta-amylase.

The function of diastase in nectar and honey has not been clearly established as nectar contains no starch or dextrins. Some authors have suggested that it may be used by the bees for digestion of starch (Crane, 1980). The major portion of diastase in honey appears to be from honeybee hypopharyngeal glands (White, 1978) and some from the plants (Crane, 1980).

2.3.6.2. Invertase

Invertases or saccharases are the most important enzymes of the oligases group. The enzymes are added to nectar by honeybee during its foraging and honey ripening and its activity continues in extracted honey unless destroyed by heating. The enzymes are aplha- glucosidase (White, 1952; White et al., 1953a), with inherent transglucosylase action. They act on sucrose hydrolysing it to glucose and fructose with six oligosaccharides intermediates the principal one being alpha-maltosyl beta-D fructofuranoside (White et al., 1953b). Maltose is formed in lesser amounts (White, 1978). The greatest amount of invertase in honey come from honeybee hypopharyngeal glands. Its presence in plant nectar pollen has not been definitely shown (White, 1978).

2.3.6.3. Glucose oxidase

Gauhe (1941) demonstrated presence of a glucose-oxiding enzyme in the hypopharyngeal glands of the honeybee which formed gluconic acid and hydrogen peroxide. This enzyme (glucose-oxidase) was later shown to be present in honey and that its production of

gluconic acid and hydrogen peroxide was responsible for the major part of the antibiotic effect of honey earlier reported (White et al., 1963b). The enzyme is virtually inactive in full-density honey but becomes active again in diluted honey. It is sensitive to visible light as well as to heat (Crane, 1980).

2.3.7. Proteins and Amino Acids in Honey

The nitrogen content of honey is low and variable. Honeys from the United States show an average nitrogen content of 0.041% with a standard deviation of 0.026 as is shown in Table 4.

Little is known of the characteristics of the protein in honey. Early work used protein contents to distinguish honey from artificial immitation mixtures and blends. Moreau (1911) noted that the common tests for proteins (Millons test, the xanthoproteic reaction, heat coagulation) showed presence of proteins in honey; and reported albumins, globulins and proteoses. Stitz (1930) found peptones, albumins, nucleo proteins and some globulin but not protamines, alcohol-soluble albumin, histones, albumoses and albuminoids.

The amino acid content and composition of honey protein varies depending on the origin of honey. The source or origin of amino acids is variable and can be the bees, nectars and pollen (Petrov, 1974). Using paper chromatography and amino acid analyser, several investigators identified up to 17 amino acids in various honeys. There is a general agreement that proline is the predominant amino acid in honey protein representing 50 - 86 % of the total amino acids (White, 1978).

2.3.8 Lipids in Honey

These occur in minute quantities in honey. Quantitative investigations of the ether-extractable lipids in cotton honey by Smith (1963) and Smith et al., (1966) showed presence of glycerides, steroids and possibly phospholipids. Using thin layer chromatography, the level of acids was established mainly as palmitic acid 27%, oleic acid 60%, and small amounts of lauric, myristoleic, stearic and linoleic acid.

2.3.9. Flavour and Aroma

The flavour of honey varies with its floral source and ranges from that reminiscent of a sugar and water syrup, to a mild aromatic, aromatic, spicy, fragrant, bitter to a strong, harsh, objectionable, rank flavour (Schuell, 1955). In most cases, the lighter the colour of honey, the milder the flavour.

Most aroma substances are derived from the flower particles or nectars. Clean extracted flower honey has aroma typical of the flower from which the nectar was collected. The essential oils characteristic to the flower species determine the aroma. In most plants, petals are the principal sources of flower aroma (Petrov, 1974).

Gas-liquid chromatographic (GLC) analysis has shown that honey aroma consists of a complex of over one hundred components (Dorrscheidt et al., 1962; Cremer et al., 1965). The main components have been found to be esters, aldehydes, ketones, alcohols and free acids with alcohols forming the principal part.

According to Cremer and Riedman (1965), certain components of honey aroma mainly methyl- and ethyl-esters of phenyl-acetic and benzoic acids form the charecteristic notes of aroma and that their absence would cause the honey to be organoleptically flat and lacking in its typical aroma. Low levels of vitamins B_1,B_2 and B_6 have also been reported to produce honey with a flat aroma (Petrov, 1974).

2.3.10. Honey colour.

The colour of honey ranges from water-white to nearly black, with variant towards tints of green or red or even blue. (Crane, 1980). Most of the substances responsible for honey colour are largely unknown. Browne (1908) noted that of the 92 honeys analyzed, 25 gave positive test for polyphenolic compounds chloride, with the most intense reactions being from dark honeys. Milum (1939) ascribed the increase in the colour of honey during storage to several factors: reactions of tannates and polyphenols with iron from containers and processing equipments, maillard reactions, caramelization and instability of fructose in acid solution. Von Fellenberg et al. (1938) later found water-soluble colouring materials to increase with increase in honey colour more than did fat-soluble colours. Helvey (1953) attributed the colour of honey to carotenoids and anthocyanins.

2.3.11. Vitamins in Honey

The levels of vitamins in honey are too low to have real nutritional significance. According to White (1978), honey has six measurable vitamins namely: riboflavin, pantothenic acid, niacin, thiamine, pyridoxine and ascorbic acid. The levels of these vitamins vary among different samples of similar honeys and among different honey types. Haydak et al . (1943) reported that commercial honey filtration reduced the vitamin content by amounts from 8 to 45%. This was ascribed to near complete removal of pollen (which is known to contain nearly all vitamins in honey) (Kitzes et al ., 1943). An additional source of vitamins in honey is the nectar (Ziegler, 1956; Luttge, 1962).

2.3.12. Hydroxymethylfurfural (HMF) in Honey

Hydroxymethylfurfural (HMF) is perhaps the most discussed minor constituent of honey. A trace of HMF is always present naturally in honey, but this rarely exceeds 10mg/kg (Duisberg et al., 1966). HMF results from the breakdown of hexose sugars such as fructose and glucose in the presence of an acid (honey is acidic pH 3.9). Metallic ions have been reported to catalyse the HMF production (Anam, 1988). HMF is considered to be of tremendous importance in honey quality because its levels are used as an indirect indicator of quality. High HMF levels indicate low quality honey.

2.3.13. Toxic substances in Honey

A few honeys and the nectar or honeydew they are derived from are toxic to man. This is probably due to a number of organic compounds which are synthesised by various plants, many with marked pharmacological activity. Therefore it is possible that such substances will find their way into the honey. It is likely that more toxic compounds are present in nectar but get into the honey in amounts not detectable by the methods used for analysis

Cases of toxic reactions arising from ingestion of certain types of honeys have been reported since antiquity, but by far the largest number is associated with the family Ericaceae (Rhododendron, Azalea, Andromeda, Kalmia spp.) (Kebler, 1896; Palmer-Jones, 1947; Howes, 1949; Carey et al ., 1959). Toxic principle associated with human poisoning include acetylandromedol landromedol (White et al ., 1959)andromedol, anhydroandromedol, and desacelyl pieristoxin B (Scott et al ., 1971), atropine from Daturia stramonium and Hyoscyamus niger (Sviderskaya, 1959) and mellitoxin from Coriaria arborea (Sutherland et al., 1947a).

2.3.14 Colloidal substances

Honey contains small amounts of colloidally dispersed substances. These are mainly made up of proteins, wax particles, pentosans and inorganic matter (Lolthrop et al., 1931). Using ultrafiltration, Paine et al. (1934) reported levels of colloidal substances to range from 0.1% in light honeys to 1% in dark honeys.

2.4. PHYSICAL PROPERTIES OF HONEY

The physical properties of honey are governed by the high concentrations of sugar that constitute most of the dry matter. Many of these properties are of great economic importance to the honey industry.

2.4.1. Rheology

When a normal Newtonian liquid flows, it is subject to internal friction, characterized by its viscosity. Although honey viscosity largely depends on moisture content, Lothrop (1939), using absolute viscosity values found great variations among honeys with the same moisture content. It has been reported that apart from moisture content, the viscosity of honey also depends on the floral source and temperature (Table 7).

Viscosity is of great importance when designing processing, pumping and draining systems for honey. According to Munro (1943), the viscosity of honey decreases on warming, most of the decrease taking place between room temperature and about 30°C.

Most honeys exhibit Newtonian behaviour but some have been shown to possess non-Newtonian characteristics. An example is heather honey which Price-Jones (1953) reported to be thixotropic to the extent that it can only be removed from the comb by a centrifugal pump when the vibrating rods effect the gel-sol transformation. This property has been ascribed to presence of high contents of certain proteins. If extracted, the heather honey protein can render clover honey thixotropic. 'Dilatancy' which is increased

viscosity with increased shear rate has been reported by Pryce-Jones (1952) to be exhibited by honeys from several Eucalyptus species. This was ascribed to their high content of the polysaccharide dextran of molecular weight in the range 1,250,000.

2.4.2. Thermal properties of Honey

Little information is available on the changes in the physical properties of honey when heated, although it is common knowledge that honey can be easily damaged by excessive heat. Helvey (1954) reported the specific heat of honey at 17.4% moisture to be 0.54 cal/gm/°C at 20°C, with a temperature coefficient of 0.02 cal/°C. White (1978) and Townsend (1954b) reported determinations of specific heats involving larger numbers of samples. These resulted in somewhat higher values as shown in Table 8. Both investigators ascribed the variations to the differences in the composition of honey .

Table 7 Viscosities of Honeys at Different Moisture Contents and Temperatures.

Type of honey (Source of honey)	Moisture content (%)	Temperature (°C)	Viscosity (Poise)
Sweet clover[a] (Melilotus)	16.1	13.7	600.0
		20.6	189.6
		29.0	68.4
		39.4	21.4
		48.1	10.7
		71.1	2.6
Sage[a] (Erigonum)	18.6	11.7	729.6
		20.2	184.8
		30.7	55.2
		40.9	19.2
		50.7	9.5
White clover[b] (Trifolium repens)	13.7	25.0	420
		14.2	269
		15.5	138
		17.1	69.0
		18.2	48.1
		19.1	34.9
		20.2	20.4
		21.5	13.6
Sage[b]	16.5	25	115
Sweet clover[b]	16.5	25	87.5
White clover[b]	16.5	25	94.0

a - Data of Munro (1943)
b - Interpolated from Munro's data.

Table 8: Specific Heat of Honey

Moisture Content (%)	Specific Heat (Cal/gm/°C)
20.4	0.60
19.8	0.62
18.8	0.64
17.6	0.62
15.8	0.60
14.5	0.56
Coarsely granulated	0.64
Finely granulated	0.73

Data of McNaughton (Townsend, 1954).

2.4.2.2. Thermal conductivity

Thermal conductivity of honey increases with decrease in moisture content and increased temperature as shown in Table 9.

Table 9. Dependency of Thermal Conductivity of Honey on Moisture Content and Temperature.

Moisture content %	Temperature (°C)	Thermal conductivity (cal/cm sec. °C)
21	2	118×10^{-5}
	21	125
	49	132
	71	138
19	2	120
	21	126
	49	134
	71	140
17	2	121
	21	128
	49	136
	71	142
15	2	123
	21	129
	49	137
	71	143

Data from Helvey (1954)

2.4.3. Hygroscopicity

Honey is a supersaturated solution with respect to glucose and fructose. In many honeys fructose content is usually higher than the glucose content. Fructose is also the most hygroscopic of all carbohydrates and this makes honey one of the most hygroscopic natural products. This property has important quality implications because; firstly honey may absorb moisture from atmosphere thus becoming diluted and more liable to yeast fermentation, and secondly honey imparts desirable softness or non-drying property to foodstuffs (White, 1957b).

Honey will gain or lose moisture from the atmosphere depending on the temperature, moisture content and relative humidity (RH) of the atmosphere. The equilibrium relative humidity (ERH) of honey, like any other product, will depend on its moisture content and the gross composition, the latter having a minor contribution. Table 10 shows the effect of the moisture content of honey on its equilibrium relative himidity.

Martin (1958) showed that moisture from the atmosphere diffuses only slowly into the honey mass. The author found that honey at 22.5% moisture when exposed to air at 86% RH for 7 days had 26% moisture on the surface layer, but there was no change 2cm below the surface. Great attention must therefore be paid to hygroscopicity during processing and handling of honey.

Table 10 Approximate Equilibrium between Relative Humidity of Air and the Water Content of Clover Honey

Moisture content (%)	Equilibrium Humidity (%)
15.9	50
16.8	55
18.3	60
20.9	65
24.2	70
28.3	75
33.1	80

Data interpolated from Martin (1958).

2.4.4. Crystallization

Most normal honeys are supersaturated with respect to glucose and fructose at normal temperatures. Part of the glucose therefore tends to crystallize and separate out, thus giving the honey the appearance of solidity. This is because as the glucose crystals form, they build up an internal network which immobilizes all the other honey constituents. Since glucose crystals are white honeys appear lighter in colour as the crystallization progresses (Dyce, 1931a).

Many pure honeys will crystallize with time after harvest. Some honeys such as that from dandelion, blue curls and ivy will even crystallize when still in the comb. A few honeys such as that

from tupelo (Nyssa) and sage have been reported not to crystallize,a property attributed to their lower glucose content compared to other honeys (Jackson et al., 1924).

Subsequent crystallization of honey after heat processing is normally associated with the introduction of glucose crystals from equipment, air and containers. Fresh honey crystallization is normally associated with the process of fine crystals naturally in the honey, although the process can also be attributed to other nucleators such as dust, pollen and fine air bubbles. These nucleators are, however, eliminated when honey is heat processed.

Predication of the tendency of honey to crystallize has been studied using two general approaches: (1) Study of model systems and (2) empirical correlation of various parameters with the observed behaviour. Using the first approach, Jackson et al.(1924) examined several system at 30°C and in particular the glucose-fructose-water ratios. The authors observed that with solid glucose hydrate, solubility decreased from 54.6% in the absence of fructose to 32.5% when fructose was present at 39.4%. The conclusion was that all honeys even those that had been reported not to crystallize were saturated at 23°C. The data, however, did not include higher concentrations of fructose which have been reported in some honeys. Lothrop (1943) extended the study to include higher fructose concentrations. It was found that there was an abrupt increase in dextrose solubility at a fructose concentration of about 150g in 100g. In the range of

solubility of 85-90g glucose per 100ml of water, the solid phase was predominantly dextrose monohydrate; while at the higher solubility range of 125-128g glucose per 100ml of water, the solid phase was mainly anhydrous glucose. The opinion was that the change in solubility of glucose was related to its degree of hydration in solution. Anhydrous dextrose is known to be more soluble than the hydrate. Kelly (1954) noted a solubility range in which anhydrous dextrose is in equilibrium with the hydrate form. It was proposed that the presence of fructose had the effect of reducing the tendency of transition from the anhydrous to the monohydrate at 50°C for solutions saturated with fructose. It was noted that most of the published information on honey relate to the zone below 30°C in which anhydrous glucose is in the solid phase. Since glucose does not normally granulate from honey until the temperature is well below 30°C, crystallization seems to occur below the transition temperature with the formation of monohydrate crystals.

Using the second approach, White et al. (1962) noted highly significant correlations between granulating tendency and the dextrose/water (D/W) ratio, of Austin (1958) giving the highest correlation. The fructose/glucose ratio commonly used earlier gave the lowest ranking index. Codounis (1962) noted that honeys with less than 30% glucose rarely granulate. The onset of crystallization is influenced by the presence or absence of nuclei, but the extent and speed are dependent on the D/W ratio. White et al. (1962) reported that D/W ratios of 1:7 and below are associated with non-crystallizing honey, and ratios of 2:1 and

above are associated with honeys that crystallise rapidly. Apart from glucose, melezitose is the only other sugar which has been reported to crystallize from honey (mainly honeydew honey), but only at high melizitose concentrations (10-25%) (Hudson et al., 1920), which are very rare. Such crystallization usually takes place in the honey comb.

2.4.5. Colloidal properties and surface tension

The colloidal materials in honey were reported by Lothrop et al., (1931) to show isoelectric point of 4.3 being positively charged at more acid pH values and negative in less acid honeys. Upon dilution, honey turbidity increases due to peptising effect of honey sugar on colloidal material and may cause flocculation if dilution is done around isoelectric point. Removal of colloidal material by flocculation with bentonite and filtration gives honey showing Tyndall effect (Paine et al., 1934).

Surface tension is a vital property of honey more so during processing as low values may result in excessive foaming and scum formation. Paine et al. (1934) noted that ultra-filtration of 25% honey solutions at 20°C gave an average increase in surface tension from 47.0 to 60.2 dyne/cm with subsequent decrease in foaming and retention of air bubbles.

2.5 HONEY PROCESSING

Honey is at its best with respect to flavour and colour soon after harvest. This naturalness should be maintained as much as possible. However, honey is not suitable for large scale marketing without processing treatment. Controlled heat treatment is usually applied to lower viscosity and thus facilitate pumping and/or straining, to melt dextrose crystals so as to delay crystallization during storage, and to destroy yeast which might cause spoilage through fermentation. Several processing approaches are used but these depend mainly on the end product which could be either liquid or solid honey. The most commonly employed method for processing honey in Kenya is, however, that described by Townsend (1973). This method is outlined in Figure 1. The method deals with production of the so called liquid honey, and its steps will be briefly explained:

2.5.1 Uncapping

'Uncaping' is the process of removing the thin wax covering the cells of ripe honey combs. This operation facilitates honey extraction.

Uncaping methods range from steam and electric operated to mechanical operations. The method used, however, depends on conditions and facilities available.

As mentioned earlier most of Kenya's honey reaches the processors already mashed and therefore uncapping is rarely part of the process.

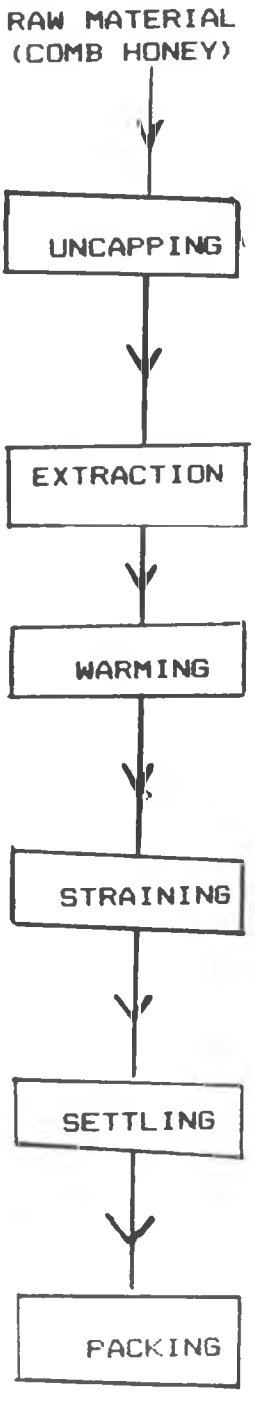

Figure I: A flow diagram for commercial honey processing (From Townsend, 1973)

2.5.2 Extraction

Honey is usually extracted from combs by centrifugal force which spins it out of the comb cells. The combs are placed in a frame basket inside a centrifuge. The number of frame baskets in each centrifuge ranpge from 2 to 50 depending on the type and scale of the equipment. Simple extraction techniques which involve gross-straining under gravity through wire meshes or coarse cloth material are widely used in Kenya as most raw honey is usually mashed. Extraction separates most combs and other extraneous matter from liquid honey which otherwise if heated together might impair the flavour of the final product.

2.5.3 Warming

This is usually done to facilitate straining. According to Townsend (1973), honey should be heated to temperatures between 38° and 43°C in order to allow optimum quantity to pass through the strainers. Most commercial processors in Kenya use tempetures between 45° and 49°C for 5 to 10 minutes as recommended by Beekeeping Branch of the Ministry of Livestock Development. Methods of heating are:

(i) Hot water coils on the sides or bottom of the extractor.

(ii) Double-jacketed sump tanks heated by either steam or electricity.

(iii) Heated corrugated pan over which the honey passes.

(iv) Double-jacketed pipes heated with water, and through which the honey passes to the strainer.

2.5.4 Straining

Straining is the process of filtering honey without the use of pressure. The operation serves to remove from honey the small wax material and other small particles.

Several types of strainers are used, but all are mainly of cloth or wire. The best strainers are those which do not stretch and maintain proper size opening. Openings of about 0.17 mm have been recommended. The Ontario Agricultural College (OAC) strainters are an example of wire mesh strainers which have been widely adopted.

2.5.5 Settling

Strained honey is usually held in settling tanks to remove air and scum. These slowly move to the surface of the honey, the air escapes and the scum can be removed mechanically. The scum consists basically of pollen, wax particles, air bubbles and other extraneous matter.

A special type of honey is processed by 'Dyce Method', which results in a semi-solid product with a fondant like texture. The honey is first heated to 49°C then strained to remove all foreign materials. This is followed by further heating to 66°C and straining. Then the honey is rapidly cooled to a temperature of 24°C or below. A 'starter' (fine, creamy, previously processed crystallized honey) is added at the rate of 10% by weight and thoroughly mixed. The honey is then filled into retail containers and held at 14°C for a week to complete the

crystallization. This process was described and patented by Dyce (1935). The end product is sold as creamed honey. This process has not been commercially exploited in Kenya.

2.6 EFFECT OF PROCESSING AND STORAGE TEMPERATURES ON HONEY QUALITY

The sugars and also some of the minor constituents of honey (mainly those responsible for colour, flavour and aroma) are damaged by high temperatures. This damange can occur either during processing at temperatures above 75°C or during storage at temperatures in excess of 27°C for long periods (Rogers, 1975). The effect of excessive processing or storage has been studied by following changes in colour, HMF, diastase activity, flavour, carbohydrate composition and extend of crystallization.

2.6.1 Colour Changes

The international price of honey is determined by its colour among other criteria. Honey darkens during processing and storage depending on its composition (fructose, moisture, acidity, etc.) and considerable variations among samples have been observed (White, 1978).

Milum (1948) found that darkening during storage depended in part upon the amount of previous darkening; hence discolouration during processing tends to reduce the subsequent rate. The author also reported that the rate of darkening increased faster as the temperature rose above 10°C. White *et al.* (1964) found that white honeys stored at 21°C darkened at the rate of 1mm

Pfund* every four months and light amber honeys at almost 3mm Pfund in the same period at the same temperature. The author concluded that dark honeys tended to be damaged by heat faster than light ones. It was pointed out by the same authors that the rate of colour deterioration was related to the rate of HMF production. Smith (1967) found that the period at a given temperature which produced 10 mm Pfund darkening in honey was of the same magnitude as the period producing HMF content of 3 mg/100g honey which agreed with calculations of White et al. (1964).

Strang et al. (1981) found no significant difference in colour after subjecting honey samples to processing temperatures ranging from 44° - 71°C for 30 minutes and flash heating to 79°C followed by immediate cooling. In a study with five Australian honeys artificially infected with Streptococcus pluton at temperatures between 10° and 80°C, Wootton et al. (1981) found that the time-temperature combinations necessary to kill the organism caused slight changes in Pfund colour grades (Table 11). Gamma radiation doses up to 25 Kilogray (KGy) did not cause significant deterioration in honey colour when Australian honeys from three floral sources were studied (Wootton et al., 1985).

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* The Pfund colour grader is the standard commercial instrument of measuring and classifying honey colour. It consists of a standard amber glass wedge with which the liquid honey is compared visually, the honey being contained in a wedge-shaped cells. The lightness or darkness of the honey is expressed by the distance in millimeters on a scale along the amber wedge whose scale is subdivided into honey colours.

Table 11: Increase in Pfund Grading (mm) for Five Honeys after Thermal Death Times (TDT) for S. pluton at Various Temperatures

		Increase in Pfund Colour Grades				
Temp (°C)	Time to Achive TDT	String Bark (71)*	Patterson's Curse (7a)*	Napunya (74)*	Spotted (48)*	Blood-wood (75)*
10	20 weeks	3	3	3	2	0
22	20 weeks	6	1	6	6	4
35	28 days	5	7	1	5	3
44	6 days	9	11	2	4	5
50	48 hours	0	b	2	0	1
60	8 hours	0	-	3	1	2
70	180 minutes	0	-	1	3	3
80	30 minutes	2	-	3	4	3

b Insufficient sample to test above 44°C

* Initial Pfund reading

Source: Wootton et al (1981)

Factors reported to affect the rate of honey darkening during processing and storage include fructose/glucose ratio (Milum, 1948), mineral content and especially copper and iron (Einset et al., 1975), colloidal constituents (Paine & Lothrop, 1933), nitrogen content, and free amino acids (Ramsay et al., 1933). According to Lynn et al. (1936), the major causes of honey dicolouration are:

(a) amino acid-aldol reactions (maillard reactions)

(b) Fructose instability (caramelisation).

(c) Combination of tannates and other polyphenols with iron salts.

Conflicting opinions exist on the relative importance of these factors. Whereas Ramsay et al. (1933) considers reactions involving nitrogenous compounds to be the major causes of honey darkening, Lynn and colleagues (1936) consider these to be of secondary importance. Ramsay and Milum (1933) found that formaldehyde prevented darkening of honey possibly by reacting with free amino groups thus preventing Maillard reactions. Wootton et al. (1976) reported the retarding effect of added sulphite further indicating that Maillard reactions might pay a major role in darkening, while addition of ascobic acid had no effect, eliminating oxidative mechanism. Of the honey sugars, fructose has been reported to be more reactive with amino acid at high temperatures (Kilp et al., 1982) and, therefore affects browning more than glucose and sucrose.

Other factors which have been reported to influence the rate of darkening in honey include moisture content and pH. Schade et al. (1958) showed that increased moisture content caused increased darkening of honey. Leo and Hannan (1949) reported an increased rate of Maillard reactions with increased pH.

2.6.2 Hydroxymethylfurfural (HMF)

The effect of excessive processing temperatures and storage time on the honey can be assessed by following the production of HMF, which is an intermediate breakdown product of monosaccharides particularly fructose and glucose. The reaction is, however, not restricted to overheated honeys only and occurs in many compounds and formulations containing sugars. It is also a common reaction during inversion of sucrose. Use is made of this fact to determine whether honey has been adulterated with commercial invert sugar, because this results in a large increase in the HMF content (Rogers, 1975).

Traces of HMF are normally found naturally in fresh honeys but these rarely exceed 10mg/kg (Duisberg et al., 1966). The presence of HMF in fresh honey has been ascribed to action of normal honey acidity (pH 3.9) on fructose at ambient temperature. By severe and prolonged storage or overheating, the HMF content may exceed the upper limit of 40mg/Kg limit for table honey, according to the Codex Alimentarius Standard (1969) for authentic normally processed and stored honeys. A HMF content of 200mg/Kg was suggested by White et al. (1980) as that beyond which honey may be considered to be possibly adulterated with invert sugar.

Based on several studies of HMF production in honey by heat and during storage, it has been shown that this area is characterized by extreme variability and that no fixed formula can be used to predict the exact effect of heating and storage on HMF content (White et al., 1980). Schade et al. (1958) found HMF to increase in four samples during storage for 13 - 15 months at 20°C; one sample showing an increase of up to 3.3 mg/100g. One California alfalfa honey was reported to have shown an increase in HMF content from 0.3 to about 7 mg/100g during 48 hours at 68°C. Hardon et al. (1960) showed the effect of holding four types of imported honeys at 50°C as indicated in Table 12. Of the 25 European honeys (most French) studied, 16 showed HMF contents equal to or less than 13 mg/100g after being held at 70°C for 10 hours (Gautier et al., 1961). Hardon and Zurcher (1962) examined the effect of holding 300 kg dums of honey at 48°C. The honey reached 48°C after 24 hours. The HMF content in three drums was reported to have increased from 1.2, 1.3, 1.2 mg/100g to 2.2, 2.7 and 2.4 mg/kg respectively after 120 hours of storage. White et al. (1964) found a linear relationship between storage temperature and the logarithm of the time required to accumulate a given amount of HMF in honey. Based on this relationship it was estimated that about 3 times the heat exposure required to obtain an HMF content of 4mg/100g honey is needed to produce a HMF level of 20mg/100g honey.

Table 12: HMF Content (mg/100g) of Honeys before and after Heating at 50°C

Source	Initial value	After 100 hrs storage	After 300 hrs storage
Guatemala	2.5	5	16
Central America	0.6	2	8
California	1.6	4.3	8
Mexico	0.1	0.8	26

Data from Hardon *et al.* (1960)

Earlier, White *et al.* (1964), subjected three honey samples to storage temperatures ranging from -20 to 60°C and determined their HMF contents. From their findings, they were able to argue that 3mg HMF per 100g honey could be produced within 18 - 36 hours, when honey was subjected to pasterurisation conditions of 63°C for 30 minutes, unless subsequent cooling was rapid. Conditions recommended for delaying crystallization (71°C for 30 minutes) could likewise cause production of 3mg HMF per 100g honey within 5 - 12 hours. It was concluded that for minimum HMF production, control of temperature is necessary and that a reduction of the storage temperature of honey by 10 - 15°F (6 - 8°C) during six months of storage will reduce HMF accumulation by one-third.

Table 13: Increases in HMF (mg/Kg) for Five Honeys after Thermal Death Time Treatments for *Streptococcus pluton* at Various Temperatures

Temp. (°C)	Time	Floral Source				
		Stringy Bark (0.6)	Patterson's Curse (221.0)	Nyapunya (13.4)	Spotted (2.5)	Blood-wood (2.5)
10	140 weeks	0.0	0.0	0.0	0.0	0.0
22	140 weeks	0.0	0.0	0.0	0.0	0.0
35	28 days	1.4	5.0	0.7	2.6	2.6
44	6 days	1.4	10.0	2.5	5.2	4.4
50	48 hours	1.9	-b	2.9	2.2	2.2
60	8 hours	2.3	-	2.9	1.0	0.0
70	180 mins	0.0	-	1.8	0.0	0.0
80	30 mins	0.3	-	0.0	0.0	0.0

b - Insufficient sample to test at 44°C

() - Initial HMF content (mg/Kg)

Source: Wootton *et al.* (1981).

Wootton et al. (1981) showed that treatment at temperatures of 10 - 80°C was unlikely to affect increase in the HMF content of honey beyond the minimum of 40mg/Kg required by the Codex Alimentarius Commission Standards (Codex Alimentarius, 1969).

Thrasyvoulou (1986) investigated the levels of the naturally occuring HMF and the change in these levels during heat processing and storage in Greek honeys. On average, an increased in HMF content of 8.8 mg/Kg and 4.1 mg/Kg for blossom and honeydew honeys, respectively after 12 months storage at room temperature (25 ± 4°C) in sealed 1 - 2 kg vials was noted. Heating at temperatures between 40° to 80°C for 1 and 5 minutes caused different rates of HMF in honeys of both same and different origins. In all cases the rate of HMF formation was found to increase with increased HMF content.

HMF content has been shown to decrease after exposure to certain forms of irradiation. Wootton et al. (1985) reported that after subjecting three honey samples to gamma-radiation from cobalt 60 at doses of 5 - 25 Kilogray (KGY) at a rate of 0.2586 KGY/min, it was found that HMF content decreased with increased irradiation doses as shown in Table 14. The opinion was that this reduction in HMF content could mask heat related deteriorations unless HMF is measured in honey prior to radiation treatment.

Several factors have been reported to affect the rate of HMF production during processing and storage. These include moisture content (Schade et al., 1958); initial HMF (Schade et al., 1958;

and Thrasyvoulou, 1986), fructose content (Kilpi et al., 1982) and metallic ions (Anam, 1988). Increase in all these factors except for pH have been associated with increased HMF production. The rate of HMF production was shown to decrease with increased pH.

Table 14: Influence of Gamma-radiation on HMF Content of Three Honeys

Radiation dose (KGY)	Floral Source		
	Stringy bark (0.5)	Napunya (14.0)	Pattersons Curse (221.0)
5	0.5	4.8	150.7
10	0	6.3	121.8
15	0	2.5	89.3
20	0	1.5	67.8
25	0	0.4	48.5

() - Initial HMF content. Standard deviation ± 3 mg/Kg HMF

Source: Wootton et al. (1985)

2.6.3 Enzyme Activity

As has been pointed out earlier, all honeys contain several enzymes, most important of which are invertase and diastase (amylases). These enzymes are destroyed if the honey is heated for too long at high temperature, or during prolonged storage. Due to its higher heat sensitivity compared to other enzymes, diastase is more often used as an indicator of overheating in

honey (Rodgers, 1975). Most honey importing countries have regulations which demand that the honey enzymes should be as intact as possible. The Codex Alimentarius Standard for honey (1969), which Kenya Standard for honey (1985) has adopted, requires a minimum diastase number of eight (8). Diastase number expresses diastase activity as millilitres of 1% starch solution that are hydrolysed by the enzyme in 1g honey in 1 hour at 40°C. However, honeys from citrus have lower enzyme contents and the Codex regulations standards, therefore, require a minimum diastase number of 3 so long as the HMF content is not greater than 15 mg/Kg.

The rate of enzyme activity varies among different honeys processed and/or stored under the same conditions. Schade et al. (1958) found an average decrease in diastase activity of 0.72% per month in honeys stored at 20°C, while White et al. (1961) showed a decline in diastase values of stored unheated honeys at room temperature (23 - 28°C) of 2.95% per month which was equivalent to a half-life of 17 months. Hardon and Zurcher (1962) found an average diastase loss of 20% and 5% for 300 Kg drums of honey held for 5 days at 48° and 43°C, respectively. White et al. (1964), based on these data, reported that the changes were relatively predictable over the temperature range of 10° - 80°C as seen in Table 15, which shows the half-life of honey diastase and invertase enzymes over this temperature range.

The authors pointed out that a reduction of 10 - 15°F (6 - 8°C) in the storage temperature for six months could reduce the enzyme loss to 1/5 - 1/6 of that at the higher temperatures.

Wootton et al. (1981) found slight changes in diastase number of five floral honeys subjected to heat treatments at 10°C for 140 days to 80°C for 30 days. Later Wootton et al. (1985) in a separate study showed that gamma irradiations using cobalt 60 at doses of 5 - 25 KGY caused insignificant losses in diastase activitities of 3 floral honeys as shown in Table 16.

Table 15: Calculated half-lifes of honey enzymes at different temperatures

Half-life (°C)	Diastase	Invertase
10	12,600 days	9,600 days
20	1,480 days	820 days
25	540 days	250 days
32.2	126 days	48 days
35	78 days	28 days
40	31 days	9.6 days
50	5.4 days	1.3 days
62.4	16 hours	3 hours
71	4.5 hours	40 mins
80	1.2 hours	8.6 mins

Source: White et al (1963a)

Table 16: Influence of Gamma - Radiation on Diastase Number of Honeys from three Floral Sources

Radiation dose (KGY)	Floral Source		
	Stringy (38)	Napunyah (10)	Patterson's curse (5)
5	32	10	5
10	32	9	5
15	29	8	4
20	29	8	4
25	31	7	3

() - Initial diastase number. Standard deviation ± 2

Data of Wootton et al. (1985).

Thrasyvoulon (1986) found an average of 59% and 40% diastase loss in one year for honeydew and blossom honey respectively stored at room temperature (25° ± 4°C).

Factors which have been suggested to affect rate of diastase loss include pH, with Schade et al. (1958) showing that the rate of inactivation is greater for honeys with low pH; and fructose content which was found by Thrasyvoulon (1986) to have positive correlation with increased rate of diastase loss. Foreign matter and toxins from such other enzymes as cholinesterases are also associated with loss in diastase activities (Pourtallier et al., 1972).

2.6.4 Flavour Changes

The honey volatiles are the major constituents to its flavour and aroma. Each floral source has a specific characteristics flavour, but this can be altered by post harvest practices including handling, processing and storage. An understanding of the influence of processing and storage on the volatile composition is of major importance in controlling flavour alterations resulting from such handling.

The storage and processing temperatures as well as the duration of exposure of honeys are considered to determine the levels of change of volatile components (Milum, 1948). According to Cremer et al. (1965), a honey sample stored for one year at room temperature showed an increase in n-pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol and n-propanol, some of the major honey volatiles. The authors were of the opinion that these substances may have originated from their corresponding amino acid (norleucine, isoleusine, leucine, alpha-aminobutyric acid, respectively). Strang and Dimick (1981) subjected alfalfa honey to processing temperatures of 44°, 49° and 71°C for 30 minutes and flash heating to 79°C, then immediate cooling. No significant difference in flavour was found among all treatments. Wootton et al. (1978) found that storage of six Australian honeys at 50°C for 44 days resulted in decrease in levels of most volatile components, especially those with honey-like aromas, but increase in levels of other compounds such as HMF, furfural and furandialdehyde.

Changes in volatiles of honey during heating or storage may arise from several causes. Both heat and acid labile components may be destroyed and others may be lost by volatilization. Also volatiles may be produced through such reactions as Maillard and caramelisation thus causing considerable changes in honey flavour (Wootton et al., 1978).

2.6.5 Carbohydrate Composition

Most changes in honey sugar take place during ripening. However, sucrose hydrolysis and subsequent production of transglycosylation may persist in the ripened honey (White, 1978). Sucrose inversion continues in fully refined honey but a greatly reduced rate.

Brown (1908) ascribed the decrease in sucrose content of honey after extraction to continued invertase activity during storage. White et al. (1961) studied the effect of storage on carbohydrate composition by comparing honey stored for two years at -20°C with those stored at room temperature (23 - 28°C), with and without pasterurisation at 55°C for 30 minutes. This storage caused the following changes: an average decrease of 18.5% of free honey monosaccharides at levels of 13% and 5.5% for glucose and fructose respectively; an increase of 69% of reducing disaccharides calculated as maltose; 13% increase in higher sugars and a relatively large increase in sucrose content. Strang et al. (1981) subjected honey samples to temperature ranges between 44° to 71°C for 30 minutes, then flash heating to 79°C and assessed their sugar content. Sucrose showed a

significant decrease at 5% level only following heating to 44°C, while fructose and glucose decreased significantly only after heat treatments at 44° and 71°C.

The changes in carbohydrate composition of honey during processing and storage have been ascribed by White et al. (1961) to enzyme activity and reversion as follows:

(a) Fructose is subject to degradation to HMF in acid medium. It could also be converted to non-reducing fructose anhydride. Fructose containing oligosaccharides may result from enzyme transfer of glucose to a fructose acceptor.

(b) Glucose - The much more disappearance of glucose than fructose could be due to specificity of the enzyme transferring glucose oligosaccharides.

(c) Reducing disaccharides are all hydrolysed by honey - glucosidase and this hydrolysis is responsible for most of the disaccharide decrease.

(d) Increase in sucrose was ascribed to mold and enzyme synthesis of sucrose by transfructosylation during storage (Edelman, 1954).

(e) Higher sugar increases are possibly due to further reversion and transglucosylation. Pigman and Guepp (1948) reported that appreciable reversion of monosaccharides in concentrated solutions in the presence of acids occurs resulting in increased disaccharide and oligosaccharide contents.

2.6.6 Crystallization

Although a natural phenomenon, honey crystallization is used as a quality criterion in international trade for various reasons:

(a) Many consumers of honey demand a product of high quality either free of crystallization or uniformly crystallised.

(b) When honey crstallizes, in most cases the surface phase has normally a higher moisture content which is suitable for attack by osmophilic yeast, hence spoilage by fermentation leading to low quality product.

Several methods for controlling and/or eliminating crystallization during processing and storage have been suggested. The most common method is pasteurisation, which serves the purposes of destroying the sugar tolerant yeast that could cause fermentation. This also melts any glucose crystals present which might act as nuclei for early crystallization. Various time-temperature combinations have been suggested for pasterurisation. Austin (1953) recommended heat treatment at 77°C for 5 minutes, followed by rapid cooling to room temperature. Townsend (1939) suggested heating at 63°C for 7.5 minutes, 69°C for 1 minute and 71°C instantaneously (about 15 seconds). Gonnet (1977) recommended a heat treatment of 78°C for 6 - 7 minutes. However, these high temperature treatments have been found to be detrimental to honey quality (White et al., 1964; Gonnet, 1977).

Another approach to control crystallization involves storage of honey at low temperatures. Austin (1953) reported that honey stored at 0°C for at least 5 weeks then at 14°C showed no granulation for 2 years. The author reported that honeys stored at 14°C without prior storage at 0°C crystallized within 5 weeks. Dyce (1931a) found that crystallization rates were greatly retarded by storage below 10°C while below 4.5°C almost no crystal growth was noticed. This was ascribed to high viscosity at the low temperatures, which reduced diffusion of glucose and thus retarded crystal formation. Dyce also found that the critical temperature for honey crystallization was 14°C. This was attributed to the balancing out of the effects of supersaturation and viscosity. This temperature (14°C) has since then been economically exploited in the so called Dyce (1935) processing method for production of creamed honey.

3.0 MATERIALS AND METHODS

3.1 SAMPLING OF CRUDE HONEY

Two honey samples of fresh crude honey were obtained from beekeepers in each of Baringo and Kitui districts. The two districts are the major sources of honey found in Kenyan markets.

The first sampling from Baringo district was done in December, 1989. The honey floral source was during this season predominantly Acacia mellifera and Acacia senegal. The second sampling was done in May 1990 when the honey was mainly from Acacia brevispica (Obiero, 1990). Sampling in Kitui district was done in February and June 1990. Information on floral source of these samples was not available, but they were all of multifloral origin, judging from the colour of the pollen grains present.

3.2 PROCESSING OF HONEY

A sample of 100 Kgs was first gross-strained by passing through a stainless steel wire mesh of 60 pores per square centimeter to remove most of the wax and foreign matter. The gross-strained sample was divided into 13 batches. One batch was analysed for its viscosity before being strained through a nylon cloth of 100 mesh which had been folded six times.

The remaining 12 batches were heated in a water bath to temperatures of 45°, 50°, 55° and 60°C for 20, 25 and 30 minutes. These temperatures were chosen on the basis that: 45°C is the temperature commonly used for honey processing in Kenya, and that

preliminary studies had shown that heating at 45°C or lower temperature for 15 minutes resulted in low straining efficiency and early crystallization of the product (Kasolia, 1989). Heating for 55°C for 30 minutes has been reported as sufficient pasteurisation without enzyme inactivation (White et al., 1961). 60°C for 30 minutes was chosen in this study was the extreme temperature-time combination which is near the recommended pasteurisation conditions of 63°C for 30 minutes by Townsend, (1973). The viscosities of the heat treated samples were determined before straining. After straining, the samples were left standing at room temperature for 4 days. The layer which had formed at the surface was scooped with a spoon each day during the holding period. The honey was then packed in 300 g glass jars fitted with plastic lids.

3.3 STORAGE STABILITY EVALUATION

One jar from each treatment was stored in a refrigerator at 4°C for analysis. The rest of the jars from each batch were divided into four groups each of 3 jars and a group stored at each of room temperature, 22°, 28° and 35°C for up to 6 months. The freshly processed honey was analysed for moisture, reducing sugars, sucrose, glucose, fructose, pH, total titratable acidity, total ash, total nitrogen, Hydroxymethylfurfural (HMF), diastase value, colour, visible browning and soluble solids.

The stored samples were visually observed every day for crystal formation. After every 2 months, one jar from each storage conditions was removed and analysed for HMF, diastase value and visible browining.

3.4 ANALYTICAL METHODS

3.4.1 Determination of Moisture Content

The refractometric method of Chataway (1932) as revised by Wedmore (1955) and as adopted by AOAC (1980) and Kenya Standards of honey (1985) was used. This involves determination of the refractive index with a refractometer at a constant temperature of around 20°C and converting the reading into moisture content (%) using a standard Table.

3.4.2 Determination of Total Reducing Sugars

The method of Lane et al. (1923) involving the reduction of a Soxhlet modification of Fehling's solution by titration at boiling point with honey solution using methylene blue as indicator was employed. This is the recommended method by FAO/WHO Codex Alimentarious (1960\9) and has also been adopted by AOAC (1980).

3.4.3 Determination of Sucrose

Sucrose content was determined by the inversion method of Walker (1917) as described by Codex Alimentarius (1969) and as adopted by Kenya Standards for honey (1985). The method involves

hydrolysis by heating with 6.34N hydrochloric acid and subsequent titration as described in 3.2.2. The sucrose content was obtained from percent invert sugar using the formula:

Sucrose content = (invert sugar content after inversion - invert sugar before inversion) x 0.95

3.4.4 Determination of Glucose and Fructose

Glucose and fructose contents were determined by the method described by Kenya Standards for honey (1985) as follows:

To 50 ml of 1% honey solution in 200 ml stoppered flask, 40 ml of 0.05N iodine solution and 25 ml of 0.1N sodium hydroxide solution are added. The flask is stoppered and kept in the dark for 20 minutes. The mixture is then acidified with 5 ml of sulphuric acid and the excess iodine is titrated quickly against a standared 0.05N sodium thiosulphate solution. A blank is prepared using 50 ml of water instead of honey solution. The glucose and fructose content are then calculated as follows:

Approximate glucose, percent (w/w) is calculated as follows:

$$W = \frac{(B - s) \times 0.004502 \times 100}{a}$$

Where B = volume of sodium thiosulphate solution required for the black (ml)

S = volume of sodium thiosulphate solution required for the sample (ml)

a = weight of the honey sample (gm)

w = approximate percent glucose

Approximate percent fructose (x) is then calculated as follows:

$$X = \frac{\text{Total reducing sugars} - \text{approximate glucose content (w)}}{0.925}$$

Actual glucose (Y) as g per 100g honey, % is calculated as:

$Y = W - 0.012\ x$ where Y is actual glucose content.

Actual fructose (Z) as g per 100g honey, % is calculated as follows:

$$Z = \frac{\text{Total reducing sugars} - Y}{0.925}$$

3.4.5 Determination of pH

pH was determined using a PYE Unicam Model 2K pH meter.

3.4.6 Determination of Total Titratable Acidity

The determination involved titration of 10g honey in 75 ml distilled water against carbonate free 0.1 N sodium hydroxide using neutralised phenophthalein as indicator, AOAC (1980). The acidity was then calculated as follows:

Acidity expressed as milliequivalents /Kg honey = 10V.

Where V = Millilitres of 0.1 N NoOH used for neutralization of 10g honey

3.4.7 Determination of Total Ash Content

Total ash content was determined by FAO/WHO Codex Alimentarius method (1969). In the method 5 - 10g of honey were weighed in a previously ignited cooled and pre-weighed silica dish and gently heated on a hot plate until the samples were black and dry, then ignited at 600°C to constant weight. The ash was cooled in dessicators and weighed. Percent ash content was calculated by the formula:

$$\text{Ash (\%)} = \frac{100\ (M_2 - m)}{(M_1 - m)}$$

Where: M_1 = weight of the dish with sample before ignition

M_2 = weight of the dish with ash

m = weight of empty dish

3.4.8 Determination of Hydroxymethulfurfural (HMF)

The photometric method of Winker (1955) using barbituric-p-toluidine was used. In the method, 5 ml of 10% p-toluidine in isopropanol containing 10% acetic acid was added to 2 ml of 20% honey solution. One millilitre of 0.5% barbituric acid was then added and the absorbance read against a blank containing water instead of barbituric acid at 550 nm. HMF was calculated as follows:

$$\text{mg/100g HMF} = \frac{\text{Absorbance} \times 19.2}{\text{Thickness of layer (Cuvette)}}$$

The results were expressed as mg HMF/kg honey.

3.4.9 Determinarion of Water-insoluble Solids

Water-insoluble solids content were determined by the gravimetric method described by Codex Alimentarious (1969). In the method, about 20g of honey is weighed and dissolved in a suitable quantity of distilled water, then filtered through a previously dried and weighed fine sintered glass crucible (pore size 5 - 40 microns) and washed thoroughly with hot distilled water (80°C) until free from sugars (Mohr test). The crucible is then dried for one hour at 135°C, cooled and weighed. The results are calculated as percent water-insoluble solids (S) as follows:

$$S = \frac{X}{W} \times 100$$

Where,

X = gain in weight of the crucible (weight of residue) in g

W = weight of the honey sample.

3.4.10 Determination of Diastase Value

Diastase activity was determined by the method of Schade et al. (1958) as modified by White et al. (1959) and Hadorn (1961) as outlined by Codex Alimentarius (1969) methods of analysis and sampling. By this method, 10 ml of 20% honey solution (containing 5 ml of 1.59 M acetate buffer of pH 5.3 and 3 ml of 0.5 M sodium chloride) is pipetted into 50 ml graduated cylinder and placed in a water bath at 40 ± 0.2°C with flask containing starch solution with a blue value 0.5 - 0.55. After 15 minutes 5 ml of starch solution was pipetted into the honey solution and

mixed. At 5 minutes intervals, 1 ml aliquots are removed and added to 10ml 0.0007 N iodine solution together with 35ml of distilled water and mixed well. Absorbance is immediately determined at 660 nm using a 1 cm cell. Taking of 1ml aliquots is continued at intervals until absorbance of less than 0.235 is obtained. The absorbance values are plotted against time (min.) on a rectilinear paper. A straight line is drawn through as many points as possible to determine the time when the reaction mixture reaches an absorbance of 0.235. The diastase value (DN) is obtained by dividing 300 by this time.

3.4.11 Determination of Total Nitrogen

The total nitrogen content of honey was determined by the Macro-Kjeldahl method (AOAC, 1980).

3.4.12 Determination of Colour

The colours of the honey samples were determined by matching with pre-determined samples from the Beekeeping Branch of the Ministry of Livestock Development-Kenya.

3.4.13 Determination of the Visible Browning

Visible browning was determined by a modified method of Hendel *et al*. (1950). In the method, 5 ml of 50% ethanol was added to 2 ml of 20% honey solution and the resultant mixture shaken thoroughly. The absorbance was read at 440 mm against a blank of 50% ethanol.

3.4.14 Determination of Viscosity

The viscosities of honey samples were determined immediately each lot attained its respective processing conditions (temperature and holding time) just before straining. The analysis were carried out with a Contraves STV Rotational Viscometer (Contraves Rheomat, AG. Zurich) using measuring system C at rotational speed II for all heated samples and measuring system D + E at rotational speed III for unheated (control) samples. The readings were converted into poises (P) values by use of system and corresponding speed provided by the manufacturer.

4.0 RESULTS AND DISCUSSION

4.1 COMPOSITION OF HONEY

The results of analysis of fresh unheated honey samples from Baringo and Kitui districts are shown in Table 17. From this table, it can be seen that the honey samples used for this study had compositions within the requirements of both Codex Alimentarins Commission (1969) and Kenya Standards for Honey (1985). The results however, show variations in several of the parameters measured. These differences were to be expected as the honeys were collected from different localities at different times of the year.

The average hydroxymethylfurfural (HMF) values of 5.1 and 7.7 mg/kg for Kitui and Baringo samples, respectively, though slightly high for fresh honey, are within the expected range for honeys from hot tropical climates. Both values were less than the 10mg/kg reported by Duisberg et al. (1966) for similar honeys.

Sum for glucose and fructose is slightly higher than the value for total reducing sugar in both samples. This has been reported to be normal by the method used for sugar determination and has been attributed to differences in reducing powers of the different sugars present in honey (AOAC, 1960).

The average total nitrogen contents of the two samples were 0.40 and 0.50 for Kitui and Baringo, respectively. Both values were higher than those reported by White et al. (1962) for American honeys. The source of nitrogen in honey is mostly the pollen incorporated, during and after harvesting.

The mean diastase activities for Kitui and Baringo honeys were 20.1 and 17.3 respectively, which are comparable to the average value of fresh honey worldwide (Vorwohl et al., 1989). A minimum diastase value of 8 units is the recommended quality standard.

4.2 CHANGES IN HONEY CHARACTERISTICS DURING PROCESSING

4.2.1 Moisture Content

Increase in holding times had very little effect on the moisture content of honey at all processing temperatures.

Increase in processing temperature resulted in decrease in moisture content from an average of 19.2% to 18.5, 18.7, 18.6 and 18.4% at 45°, 50°, 55° and 60°C respectively for Kitui honey, while for Baringo honey, the decreases were from an average of 17.6% to 17.3, 17.2, 17.2 and 16.9%, respectively, for the same temperatures. Although these decreases were not significantly different ($P = 0.05$), they are important from the point of view of honey keeping quality.

Table 17: Mean Physico-chemical Composition of Fresh Kitui and Baringo Honeys

Component	Mean Composition	
	Kitui Honey	Baringo Honey
Moisture %	19.2	17.6
Total reduring sugars %	75.8	76.5
Sucrose %	1.4	2.85
Glucose %	36.8	39.5
Fructose %	41.9	43.8
Total acid meq/kg	28.0	27.4
pH	3.9	3.9
Total nitrogen %	0.4	0.5
Hydroxymethylfufural (mg/kg)	5.1	7.7
Diastase value	20.1	17.3
Total water insoluble solids %	0.12	0.18
Total Ash %	0.36	0.25
Colour-(A_{440})	0.314	0.20
Viscosity (P)	41.69	59.75

Honeys with moisture contents of above 20% are liable to fermentation by osmophilic yeasts. Such honeys can, however, have the moisture contents lowered by heat treatment, thereby reducing the possibility of fermentation. An example in this study was the Baringo honey whose water content was reduced by processing at 60°C from an initial average value of 17.6%, a level likely to promote fermentation even when yeast counts are less than 1000 cells per gram, to 16.9% which was just below the 17.1% moisture content reported by Lochhead (1933) as that below which fermentation is inhibited. Reduction of moisture content of honey by heat processing is also likely to influence the rate of honey crystallization, especially because the glucose/water index of White et al. (1962) is increased. The author reports the ratio of 1.8 as that below which crystallization is inhibited, 2.1 as the value associated with high rates of crystallization.

4.2.2 Sugar Composition

There were small variations in total reducing sugars, sucrose and glucose indicating that heat treatments had little effect on these sugars. The effect of temperature - time heat treatement on fructose contents are shown in Table 18a. Increase in holding time caused insignificant differences ($P = 0.05$) in fructose content at temperature levels used in this study.

The effect of temperature on fructose content is shown in Table 18b. For Kitui honey, there were significant differences ($P < 0.05$) between the fructose levels of the honey heated at 45°C and

the levels of those heated at 55° and 60°C. As for Baringo samples, significant differences ($P < 0.05$) occurred between fructose values of the honey heated at 45°C and those heated at 55° and 60°C. There were significant differences ($P = 0.05$) between fructose levels of the honey heated at 50°C and those heated at 55° and 60°C (Table 18). These results show that the sensitivity of fructose to heat increased with increase in temperatures.

The faster disappearance of fructose than other sugars during heat processing could be attributed to its more relative ease than glucose to react with amino acids at high temperatures and its preference to glucose in formation of HMF when heated in an acid medium (honey is acidic) (Kilpi, 1982). It could also be due to higher rate of caramelisation compared to that of glucose or sucrose (Hyvonen et al., 1981a; Hyvonen et al., 1981b). Variation in holding times did not have any significant ($P = 0.05$) effect on the general sugar composition.

4.2.3 pH and Total Titratable Acidity

The heat treatment had no significant effect on the pH values of honeys used in this investigation.

Table 18a: Mean Fructose Contents of Kitui and Baringo Honeys after Heat Treatment at Different Temperatures and Holding Times

Processing Temperature (°C)	Holding Time (Mins)	Total Fructose (%)	
		Kitui	Baringo
Unheated	-	42.9	43.8
45	20	41.9	42.7
	25	41.7	42.6
	30	42.1	42.5
50	20	42.1	42.3
	25	41.4	42.8
	30	40.3	43.0
55	20	41.1	41.7
	25	41.0	41.5
	30	40.9	41.8
60	20	40.7	40.8
	25	38.5	42.3
	30	40.5	41.7

Table 18b: Mean* Fructose Contents (%) of Kitui and Baringo Honeys after Heat Treatment at Different Temperatures for 20, 25 and 30 Minutes[1]

Temperature (°C)	Total Fructose	
	Kitui	Baringo
45	41.7^{b}	42.6^{b}
50	$41.3^{a,b}$	42.7^{b}
55	41.0^{a}	41.7^{a}
60	39.9^{a}	41.6^{a}

* Means followed by the same letter in each column are not significantly different at 5% level.

1 Effect of holding time was not significant (P = 0.05) hence taken as average for each temperature level.

Standard Errors (SE) are 0.4006, 0.4676, 0.3138, 0.5186 for Kitui and 0.1447, 0.2603, 0.0764, 0.3393 for Baringo honeys at 45°, 50°, 55° and 60°C, respectively.

The total acid content of Kitui and Baringo honeys showed no significant difference at 5% level between all the temperature - time combinations used for this study. Heat treatment did not therefore affect the acidity of the honey samples studied.

4.2.4 Total Ash

Means of total ash contents of Kitui honey ranged between 0.36 - 0.37%, while those of Baringo honeys ranged between 0.28 - 0.30% honeys after heat treatment. The differences between the means were, however, not significant (P = 0.05). The slight increase in ash contents from the initial mean values (Kitui - 0.36%, Baringo - 0.28%) could be due to corresponding decrease in moisture contents of the honeys during heat treatment.

4.2.5 Hydroxymethylfurfural (HMF)

The effect of processing temperature and time on HMF levels are shown in Table 19a. Data from this Table shows that there was generally increased HMF accumulation with increased processing temperature and time. The average HMF values ranged from 10.8mg/kg at 45°C for 20 minutes to 15.1 mg/kg at 60°C for 25 minutes for Baringo honey. Levels for Kitui honey ranged from 11.6 mg at 45°C for 25 minutes to 14.9 mg/kg at 60°C for 25 minutes.

The increase in average HMF due to increase in processing time was small for both Kitui and Baringo and not significant (P = 0.05).

Table 19a: Mean Hydroxymethulfurfural (HMF) of Kitui and Baringo Honeys after Heat Treatment at Different Temperatures and Holding Times

Processing Temperature (°C)	Holding Time (Mins)	HMF CONTENT (mg/kg) (%)	
		Kitui	Baringo
Unheated	-	5.1	7.7
45	20	11.9	10.7
	25	11.6	12.2
	30	12.1	12.1
50	20	12.4	12.4
	25	12.9	12.3
	30	13.4	13.0
55	20	14.2	14.7
	25	14.2	14.6
	30	14.2	14.8
60	20	14.8	14.5
	25	14.9	15.1
	30	14.6	14.9

The effect of temperature on mean HMF production is shown in Table 19b. For Kitui honey, there were significant differences (P = 0.05) between the levels of the honeys heated at 45°C and those heated at the rest of the temperatures. Also honeys heated at 50°C showed significant differences (P = 0.05) with respect to HMF accumulation than those heated at 55° and 60°C. There was no significant difference with respect to HMF accumulation between heat treatment at 55° and 60°C for Kitui honey.

For Baringo honey, analysis of variance showed no significant differences (P = 0.05) in HMF accumulation among honeys heated at 45°, 50°, 55° and 60°C although the mean range of 11.7 mg at 45°C to 14.9 mg/ kg at 60°C was similar to that of Kitui honey (Table 19a). This can be attributed to the species and seasonal differences between the two Baringo samples as already reported for 3.1. This is further explained by differences for their variation coefficient which were 7.4% for Kitui and 24.8% for Baringo honeys.

Increased HMF production has been associated with decreased fructose content of similar honey sample (Kilpi, 1982) under similar processing conditions. Results of this study support these earlier findings. The temperature - time combinations used for processing in this study resulted in accumulation to maximum levels of HMF of 14.7 mg/kg for Kitui and 14.9 mg/kg for Baringo samples at 60°C (Table 19). Both of these values were, however,

Table 19b: Effect of Processing Temperatures on Average* HMF Accumulation of Kitui and Baringo Honeys During Heating at 20, 25 and 30 Min[1].

Temperature (°C)	HMF Content (mg/kg)	
	Kitui	Baringo
45	11.9	11.7[a]
50	12.9	12.8[a]
55	14.2[a]	14.7[a]
60	14.7[a]	14.9[a]

* Means followed by the same letter in each column are not significantly different at 5% level.

1 The differences in HMF increase due to above holding times were negligeable hence taken as average.

SE are 0.3894, 0.3687, 0.2162, 0.3416 for Kitui and 0.6853, 0.8162, 1.4731, 1.2025 for Baringo honeys at 45°, 50°, 55° and 60°C, respectively.

well below the maximum of 40 mg/kg recommended by each of both Codex Alimentarins Commission (1969) and Kenya Standards for Honey (1985).

4.2.6 Total Water-Insoluble Solids

There were only minor changes in total water-insoluble solids contents of both Kitui and Baringo honeys. These variations were, not significant (P = 0.05) meaning that the heat treatments used had only little effect on this parameter.

4.2.7 Diastase Activity

Some decrease in diastase number occurred following heat treatment at all temperature-time combinations. The final diastase levels were, however, not significantly different (P = 0.05) from the initial levels. The effect of heat treatment on honey quality in this case was therefore of minor importance. Results of this study are in agreement with those of Wootton et al. (1981).

Low diastase activities are associated with overheating during processing and/or storage of honey for long periods at high temperatures. In this study the temperature-time combinations used reduced the diastase activity to minimum diastase numbers of 19.31 for Kitui honey at 60°C for 30 minutes and 17.02 for Baringo honey at 60°C for 20 minutes. The two values were, however, well above the diastase number of 8 recommended by Codex Alumentarius Commission (1969) and Kenya Standards for Honey

(1985). The effect of heat treatment on honey quality based on processing temperature-time combinations used in this study was therefore of very minor importance.

4.2.8 Nitrogen

Processing temperature and time combinations used in this study had no significant effect on nitrogen content of the honeys. The slight increases observed in nitrogen content could be due to the concentration effect resulting from decreases in moisture during processing.

4.2.9 Colour and Visible Browning

The colour of honey was expressed in terms of its optimal density at 440 nm. Differences between the values of honeys heated at 45°C and those heated at the other processing temperatures were small and not significant ($P = 0.05$). There was also no discernible visual colour differences, although all the honeys had a cloudy appearance after heat processing. The results of this study agree with those of earlier investigators (Strang et al., 1981; Wootton et al., 1981). A marked difference in visual colour was, however, observed between the unheated and the heated samples from both the Kitui and the Baringo honeys as shown in Table 20a. There were also significant differences ($P = 0.05$) among the optical densities of the unheated and all heated honey samples from the two districts (Table 20b).

4.2.10 Viscosity

Average viscosity of unheated honeys, measured at the shear rates of 190s^{-1} has been shown in Table 17. The change in the viscosity of the honeys during processing were measured at the shear rate of 24.9s^{-1} These values are shown in Table 21. The viscosity of the unheated Kitui honey dropped sharply from 41.69 to 10.1 poises (P) on heating to 45°C for 30 minutes then thereafter dropped gradually to 6.77P for sample heated at 60°C for 30 minutes. The viscosity of the Baringo honey on the other hand dropped from 59.75 to 20.35P due to heating at 45°C for 30 minutes and dropped gradually thereafter to reach 10.85P for honey heated at 60°C for 30 minutes.

The effect of temperature on viscosity changes is shown in Figure 2 for Kitui and Baringo honeys. For Kitui honey, there were significant differences ($P = 0.05$) among the viscosity of the honeys heated at 45°C and those heated at all other temperatures. There were also significant differences ($P = 0.05$) among the honeys heated at 50°C and those heated at both 55° and 60°C. This trend was followed by the Baringo honeys with samples showing significant differences ($P = 0.05$) between 45°C heat treatment and the other three temperatures. A significant difference in viscosity also occurred between honey heated at 50° and 60°C ($P = 0.05$).

The effect of holding time on honey viscosity was not significant ($P = 0.05$) at processing temperature levels used.

Table 20a: Effect of Heat Treatment at Different Temperature Time Combinations on Colour Changes of Kitui and Baringo Honeys

Processing Temperature (°C)	Holding Time (Mins)	COLOUR CHANGES			
		KITUI 1	KITUI 2	BARINGO I	BARINGO 2
Unheated	-	Light amber	Amber	Extra White	Light amber
45	20	Amber	Amber	Light amber	Amber
	25	"	"	"	"
	30	"	"	"	"
50	20	Amber	Amber	Light amber	Amber
	25	"	"	"	"
	30	"	"	"	"
55	20	Amber	Amber	Light amber	Amber
	25	"	"	"	"
	30	"	"	"	"
60	20	Amber	Amber	Light amber	Amber
	25	"	"	"	"
	30	"	"	"	"

Table 20b: Effect of Processing Temperature-Time Combination on Mean (two replications) Optical Density of Kitui and Baringo Honeys after Heat Treatment at Different Temperatures and Holding Time.

Processing Temperature (°C)	Holding Time (Mins)	OPTICAL DENSITY (440nm)	
		Kitui	Baringo
Unheated	-	0.31	0.20
45	20	0.39	0.23
	25	0.38	0.23
	30	0.38	0.23
50	20	0.39	0.23
	25	0.39	0.23
	30	0.40	0.24
55	20	0.40	0.25
	25	0.40	0.25
	30	0.40	0.25
60	20	0.40	0.23
	25	0.40	0.25
	30	0.41	0.25

Table 21: Mean Viscosities of Kitui and Baringo Honeys after Heat Treatment at Different Temperature-Time Combinations

Processing Temperature (°C)	Holding Time (Mins)	VISCOSITY (POISES)	
		Kitui	Baringo
Unheated	-	41.7	59.8
45	20	11.5	24.8
	25	10.1	21.2
	30	10.1	20.4
50	20	9.3	17.8
	25	8.7	17.7
	30	8.8	17.1
55	20	7.9	14.8
	25	7.8	13.8
	30	7.4	12.7
60	20	7.1	11.6
	25	6.9	11.2
	30	6.8	10.9

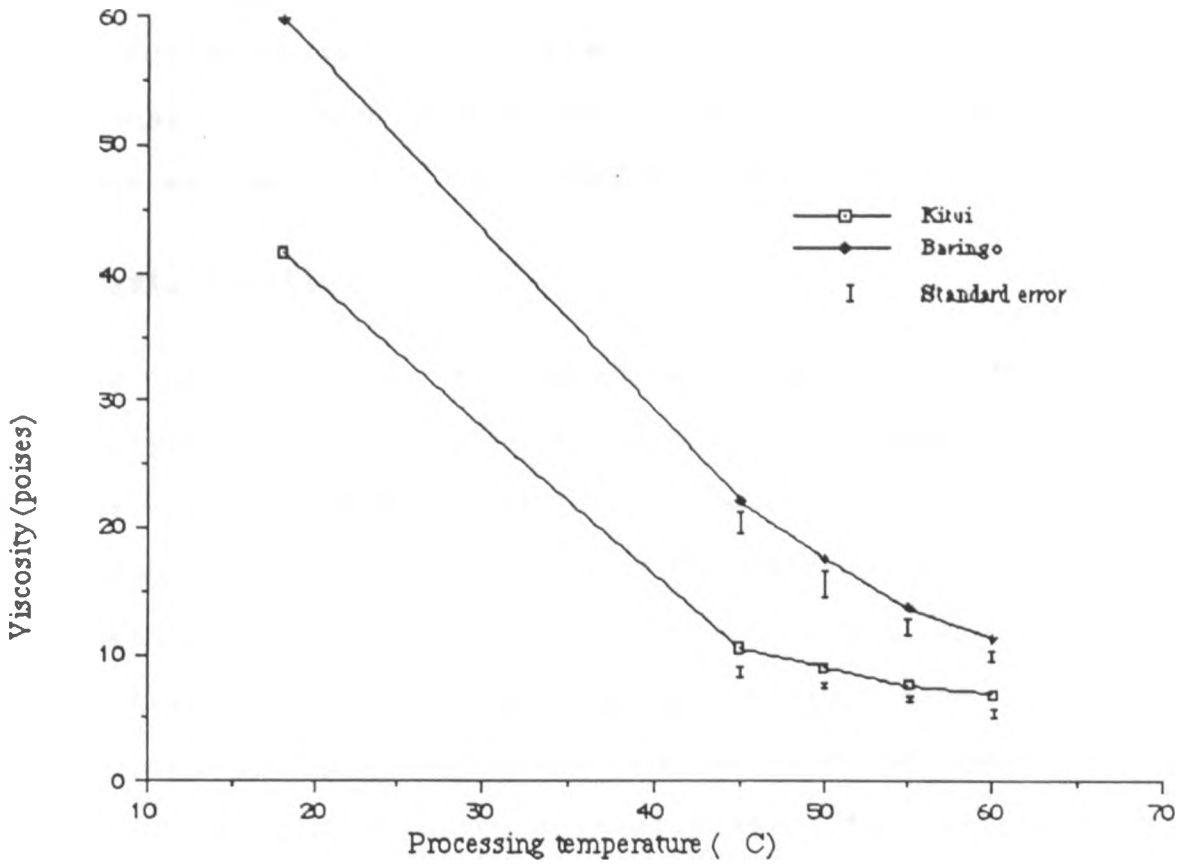

Figure 2: Effect of processing temperature on mean* viscosity (poises) of Kitui and Baringo honeys for 20, 25 and 30 min.

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*Effect of holding time was not significant (p=0.05).

The significance of honey viscosity in the rate of straining (expressed as time taken for a given quantity of honey to pass through the first fold of straining material), and percentage yield recovery as indicated in Table 22. From Table 22 it can be observed that straining rate and percentage recovery were lower at low temperatures than at high temperatures.

4.2.11 Crystallization

The effect of processing temperature and time combinations on rates of crystallization of the processed honey samples during storage at room temperature (18±4) is shown in Table 23a. The data in Table 23a show that increase in holding time at any given processing temperature resulted in very minor reductions in rate of crystallization. Varying processing time for a particular processing temperature did not have a significant effect ($P = 0.05$) in delaying crystallization for all samples tested. The effect of processing temperature on increase in the delay in crystallization of Kitui and Baringo honeys is shown in Table 23b. From Table 23b it can be seen that increased processing temperature resulted in increased delay in crystallization for samples studied.

Analysis of variance showed that the increase in delay in crystallization was significant ($P = 0.05$) among all temperature levels used.

Table 22: Mean Straining Times and Yield Recoveries of Kitui and Baringo Honeys at Various Processing Temperatures*.

Temperature (°C)	Recovery %		Straining Time	
	Kitui	Baringo	Kitui	Baringo
18 (unheated - control)	55.8	43.3	>24 hrs	>24 hrs
45	95.5[a]	93.7	28 mins	34 mins
50	96.0[a]	96.3	21 mins	18 mins
55	98.3[b]	97.5[a]	5 mins[a]	7 mins[a]
60	98.6[b]	98.0[a]	3 mins[a]	5 mins[a]

* Means followed by the same letter in each column are not significantly different (P = 0.05)

Table 23a: Effect of Processing Temperature on Crystallization of Kitui and Baringo Honeys after Heat Treatment at Different Temperatures and Holding Time.

Processing Temperature (°C)	Holding Time (Mins)	Days to First Visible Crystallization			
		Kitui 1	Kitui 2	Baringo I	Baringo 2
Unheated	-	5	27	4	8
45	20	10	48	15	32
	25	10	48	17	36
	30	12	52	17	39
50	20	24	60	40	51
	25	24	66	44	56
	30	27	64	40	54
55	20	48	73	63	69
	25	53	73	71	86
	30	51	76	81	81
60	20	71	84	90	126
	25	64	82	90	164
	30	70	84	105	160

Table 23b: Effect of Processing Temperature on Crystallization of Kitui and Baringo Honeys

Processing Temperature (°C)	Days to First Visible Crystallization			
	Kitui 1	Kitui 2	Baringo 1	Baringo 2
(unheated 18±4°C)	5	27	4	8
45	11	49	16	18
50	25	63	41	29
55	51	74	72	53
60	68	83	95	71

S.E are 0.0085, 0.0082, 0.0079, 0.0094 for Kitui and 0.0990, 0.0940, 0.0160, 0.076 for Baringo honeys at 45°, 50°, 55° and 60°C, respectively.

The decreased rate of crystallization with increased processing temperature can be explained by the fact that the higher the temperature for a given process time, the higher the number of molecules of the the crystallizing form of glucose monohydrate are transformed to the more soluble form of anhydrous glucose. This is in accordance with Lothrop's (1943) and Kelly's (1954) models of tendency of honey to crystallize.

From Tables 23a and b, it can also be seen that there were variations in rates of crystallization between samples from the two districts and also within samples from the same district. These differences could be due to variations in chemical and physical compositions occuring as a result of species, seasonal and locational differences of honeys. Use of glucose/water index of White et al. (1962) to explain the observed variations in crystallization rate in this case will, however, be misleading as the glucose levels were estimates which include reducing disaccharides such as maltose which have been reported to range between 2.74 - 15.98% of total honey composition (White et al., 1962).

4.3. EFFECT OF STORAGE TEMPERATURE AND TIME ON CHANGES IN HMF, DIASTASE, COLOUR AND CYSTALLIZATION OF HONEY

4.3.1 Changes in HMF Contents

The mean HMF contents of Kitui and Baringo honeys during storage at various temperatures are shown in Table 24. There were noticeable variations in HMF accumulation at a given storage temperature with time, dependent on the differences in processing temperature/time combinations. These changes were, however, not significant ($P = 0.05$). From Table 24 it can be observed that there were marked variations in HMF content of both Kitui and Baringo honey samples at all the storage temperatures and/or time (months). The rates of HMF changes were different between Kitui and Baringo samples. These observations are in agreement with those of earlier investigators who reported differences in rates of HMF production between honey from different regional, botanical and seasonal sources (Shade et al., 1958; White et al., 1980 and Thrasyvoulon, 1986). The rate of HMF accumulation increased highly significantly with increased storage temperature. These rates were in the order of 0.048, 0.079, 0.360 and 2.156 per month for Kitui and 0.064, 0.094, 0.402 and 2.542 per month for Baringo samples at 18°, 22°, 28° and 35°C respectively.

Table 24: Mean Hydroxymethylfufural (HMF) Contents (mg/kg) of Processed Kitui and Baringo Honeys During Storage at Different Temperatures

Storage Time (Months)	Storage Temp (°C)	Kitui Honey	Baringo Honey
Initial		13.4	13.5
	Room (18±4)	14.4 ± 0.64	14.0 ± 0.70
2	22	15.9 ± 0.64	15.2 ± 0.79
	28	22.5 ± 1.37	23.7 ± 1.42
	35	49.7 ± 4.71	70.2 ± 2.30
	18	15.3 ± 0.69	15.2 ± 0.80
4	22	17.3 ± 0.70	17.0 ± 0.80
	28	32.6 ± 2.39	34.1 ± 2.29
	35	115.2 ± 8.70	137.6 ± 3.08
	18	16.1 ± 0.71	16.7 ± 0.80
6	22	19.7 ± 0.70	18.8 ± 0.90
	28	49.7 ± 3.60	48.2 ± 1.15
	35	270.9 ± 21.64	255.8 ± 3.17

Analysis of variance showed that HMF content for samples stored at 35°C were significantly different at 5% level from the HMF contents of all the honeys under other storage conditions for both Kitui and Baringo honeys within the storage period considered. The results also showed that the Baringo sample stored at 28°C accumulated HMF at a rate significantly different ($P = 0.05$) from those at 22° and room temperature. The values from the last two storage temperatures were not statistically different from each other. For Kitui, the sample stored at 22°C was not significantly different ($P = 0.05$) in its mean HMF content from those stored at both room temperature and 28°C. However, the HMF values at the last two storage temperatures were found to be statistically different ($P = 0.05$) from each other. Figure 3 shows the increases in HMF content of Kitui and Baringo honeys after 6 months storage at various temperatures. From this Figure and Table 24 it can be seen that storage at higher temperatures i.e. 28 and 35°C resulted in honeys with HMF values above those recommended for table honeys after 6 months storage. Analysis of variance for Baringo samples showed significant differences ($P = 0.05$) in HMF contents at all storage times considered i.e. 0, 2, 4 and 6 months. Kitui honey showed significant differences in HMF contents after storage for 6 months and the rest of storage times considered. The HMF levels after storage for 2 and 4 months did not however, differ significantly ($P = 0.05$) from each other at all the storage temperatures considered.

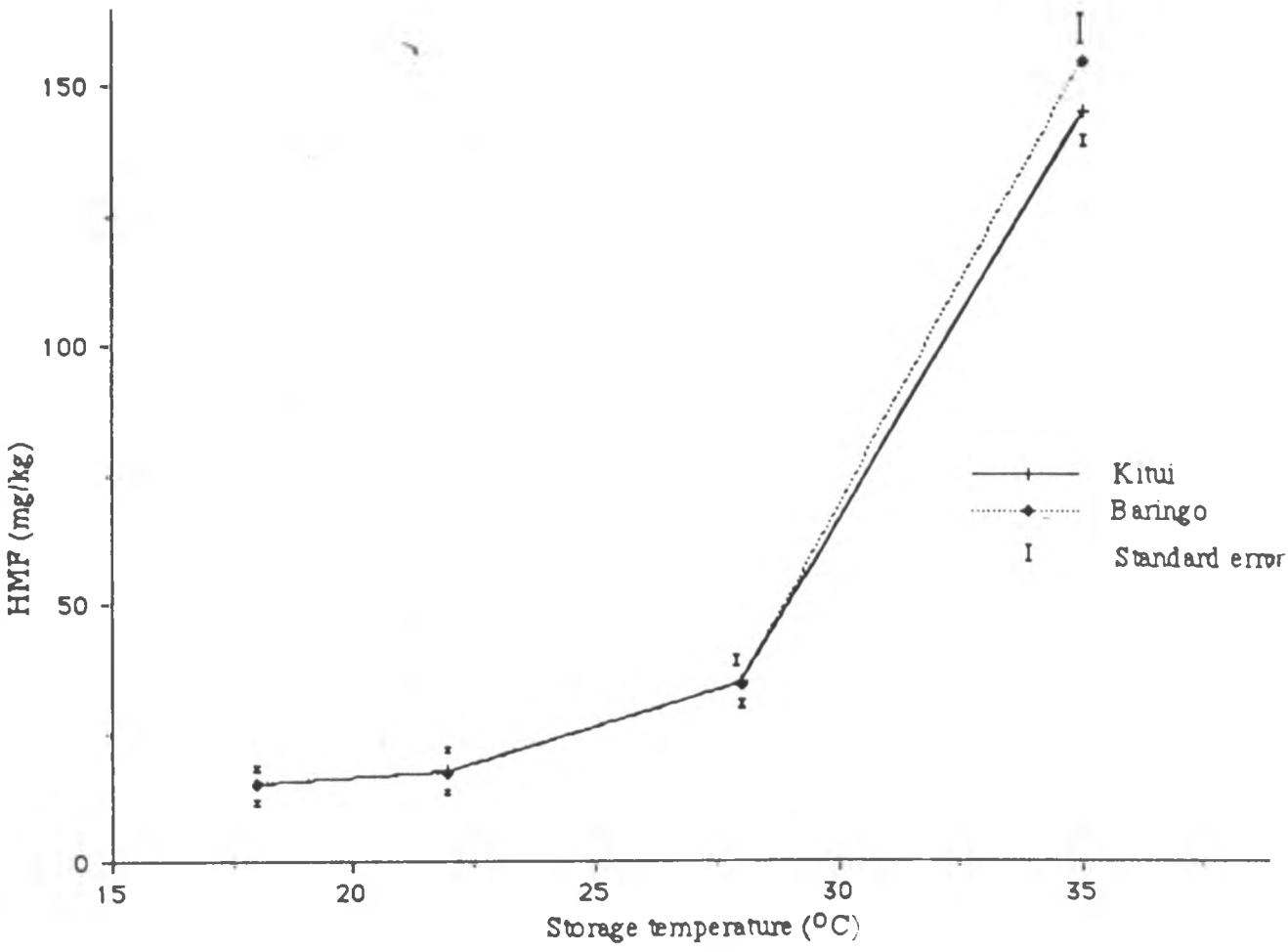

Figure 3: Effect of storage temperature on mean hydroxymethylfurfural (HMF-mg/kg) accumulation of processed Kitui and Baringo honeys after 6 months.

These results indicate that the Baringo honey produced HMF at higher rates than Kitui honey during storage. The reason for this could be probably due to higher fructose contents of Baringo than of Kitui honeys as reported by Kilpi et al., (1982). This could also be associated with higher initial HMF contents in Baringo than in Kitui honey similar to what had been reported by Schade et al. (1958) and Thrasyvoulou, (1986).

Figure 4 shows a plot of data of Table 24 and the Exponential Growth Law $P_o e^{rt} = P$ (Barnett et al., 1982).

Where P_o = Population at t = 0

e = 2.7182818

t = time

r = monthly rate of change

P = population at time t

The purpose of this plot was to try and estimate the time taken to increase the HMF levels of a sample from an initial value to the maximum of 40 ppm allowed by Codex Alimentarins Standards (1969) and the Kenya Standards for Honey (1985). From this graph it could be deduced that honey stored at 35°C could only keep for 1.7 and 1.5 months for Kitui and Baringo samples, respectively. At the lower temperature of 18±4°C the honey would keep for 77 and 58 months for Kitui and Baringo samples respectively.

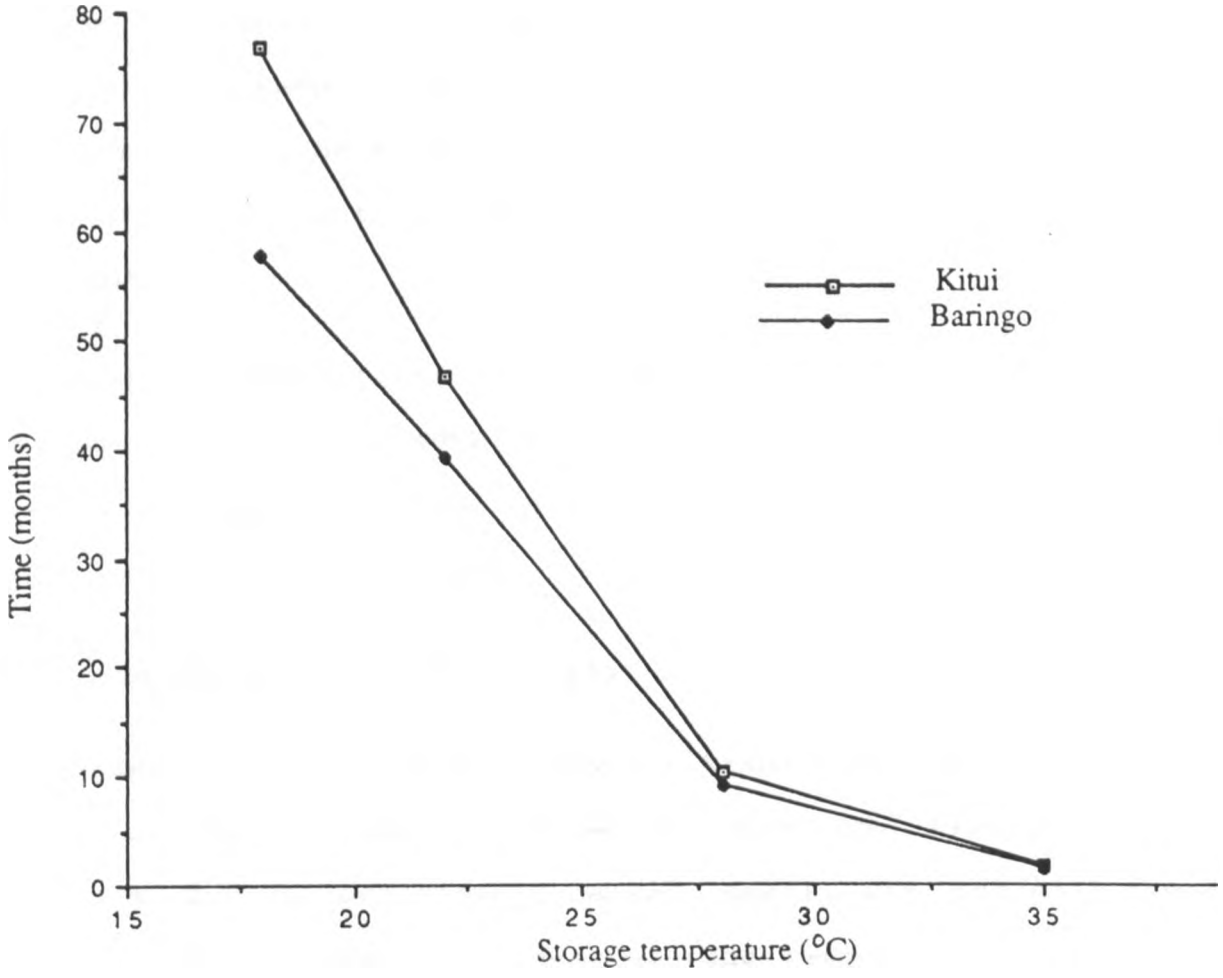

Figure 4: Effect of storage temperature on time taken to accumulate 40 mg/kg of HMF in processed Kitui and Baringo honeys.

From this information it can be argued that table honeys stored in hot climates e.g. North Eastern Province and parts of Coast Province of Kenya with average daily temperature of 28°C and above can only keep for a maximum of about 10 months, while the same honeys can be kept for at least 77 months in the cool regions of the country, where average daily temperatures are about 14°C.

The joint recommendation of Ministries of Health and Livestock Development of Kenya (1988) for maximum storage of honey is 2 years. From Figure 4 it can be calculated that only honeys kept at or below 25°-26°C will keep for this long.

4.3.2 Changes in Diastase Activity

The losses in diastase activity for Kitui and Baringo honey samples during storage are shown in Table 25. Previous heat treatments during processing, caused small and insignificant variations in diastase number over storage temperature and time. Baringo honey on average lost more diastase activity than Kitui honey, but the differences were not significant ($P = 0.05$). However, the trends of losing the enzyme activity were similar for both samples. This is in accordance with earlier investigations on effect of storage conditions on loss of diastase activity (Schade et al., 1958; White et al., 1961; Wootton et al., 1981; Thrasyvoulon, 1986). The rates of enzyme denaturation increased with increased storage temperature and differed slightly among different samples, as is shown in Table 26.

Table 25: Mean Diastase Number for Kitui and Baringo Honeys During Storage

Storage Time (Days)	Average Storage Temp (°C)	Mean Diastase Number	
		Kitui Honey	Baringo Honey
Initial		20.1	17.3
	Room (18)	19.72 ± 0.47	16.82 ± 0.41
2	22	18.84 ± 0.47	16.66 ± 0.67
	28	17.96 ± 0.19	14.59 ± 0.45
	35	12.22 ± 0.19	10.39 ± 0.42
	18	18.87 ± 0.33	16.22 ± 0.46
4	22	18.27 ± 0.29	15.92 ± 0.50
	28	15.91 ± 0.14	12.78 ± 0.37
	35	6.06 ± 0.14	6.55 ± 0.19
	18	18.4 ± 0.33	15.8 ± 0.50
6	22	17.3 ± 0.28	15.4 ± 0.55
	28	14.0 ± 0.10	10.5 ± 0.15
	35	4.0 ± 0.10	4.7 ± 0.15

Table 26: Rate Constants (K)* for Diastase Inactivation of Kitui and Baringo Honeys

Temperature of Inactivation (°C)	Rate of Inactivation Coefficient	
	Kitui Honey	Baringo Honey
18	0.000476	0.000493
22	0.000855	0.000627
28	0.001926	0.002604
35	0.008289	0.007258

* Calculated from the first order equation

$$K = \frac{1}{t} \ln \frac{100}{x}$$

Where: K = Rate constant

t = time in days

x = percentage activity remaining after time t

The residual diastase activities in all samples after 6 months of storage were significantly different (P = 0.05) from each other for both Kitui and Baringo honeys.

Kitui samples showed significant differences (P = 0.05) in diastase numbers among all storage temperatures studied, while Baringo honeys showed significant differences (P = 0.05) diastase number among samples stored at 35°C and those stored at the rest of the temperatures. There were also significant differences between the honey stored at 28°C and those stored at each of 18 and 22°C. The effects of storage temperatures on changes in diastase activity after 6 months are shown in Figure 5. White et al. (1964) had reported that a reduction of 6° - 8°C in the storage temperature for six months can result in reduction of enzyme loss by 1/5 - 1/6. The present study disagrees with this finding.

Using the data of Tables 25 and 26 and the exponential decay equation $Poe^{-rt} = P$ (Barnett et al., 1982),

Where Po = Population at t = 0,

e = time

r = daily rate of change

p = population at time t

a plot was made to estimate the time in months required to reach the minimum diastase number of 8 recommended by both Codex Alimentarius (1969) and the Kenya Standards for Honey (1985) at various temperatures. This plot is shown in Figure 6. From the

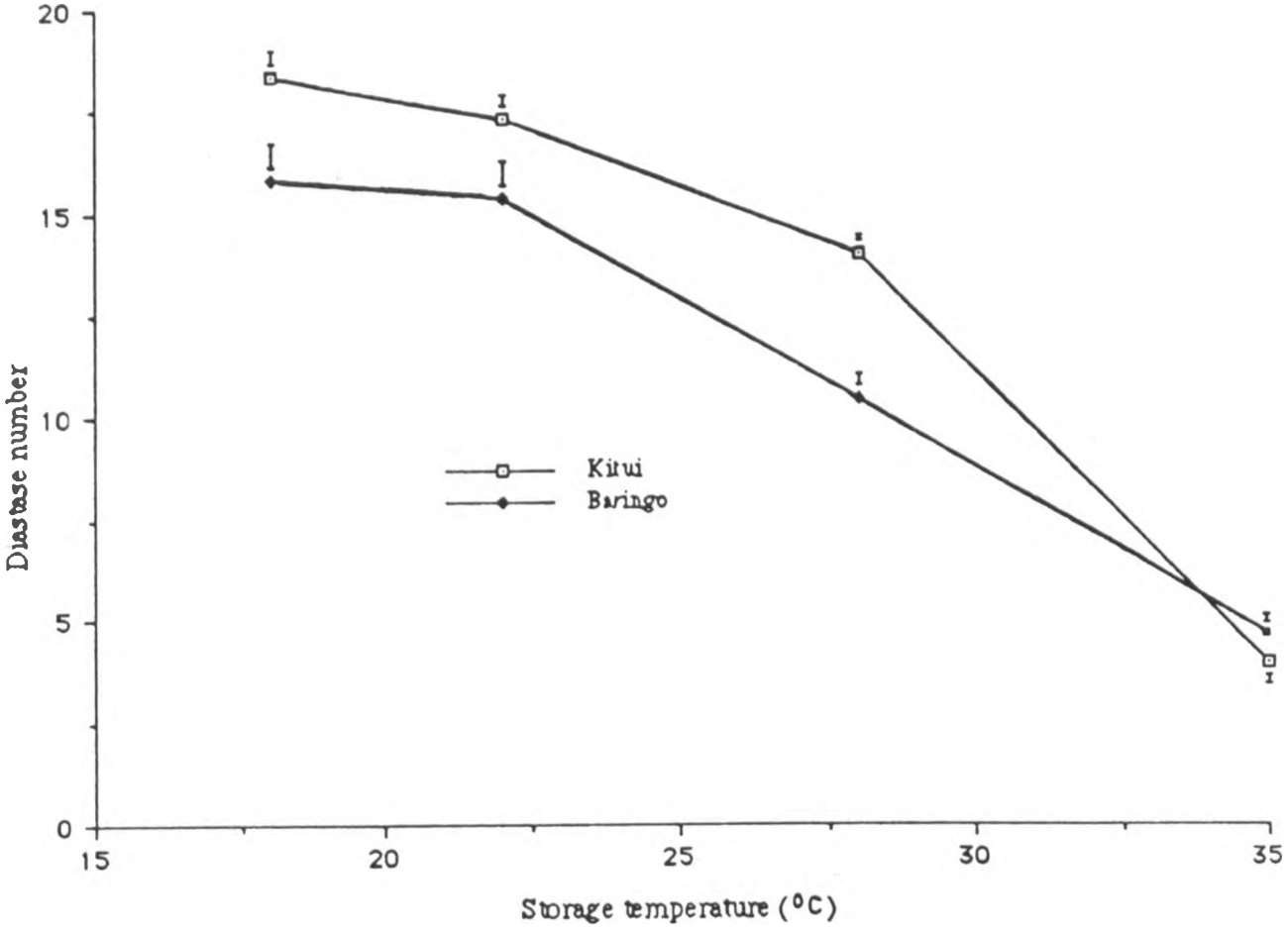

Figure 5: Effect of storage temperature on mean diastase activity (Diastase Number) of processed Kitui and Baringo honeys after 6 months.

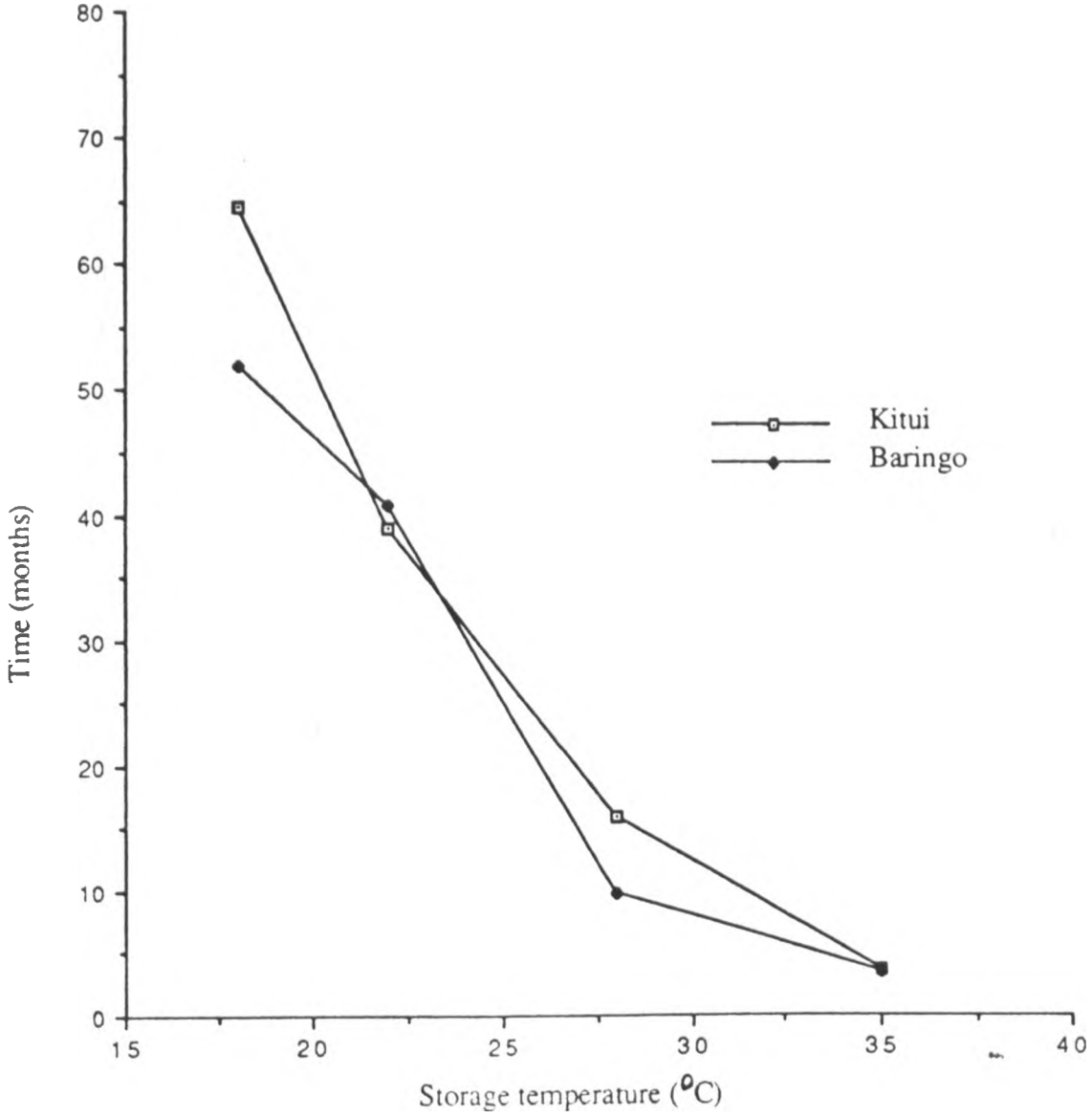

Figure 6: Effect of storage temperature on time taken to inactivate diastase activity to Diastase Number 8 in processed Kitui and Baringo honeys.

Figure it can be seen that honey can keep for several years (about 5 and 4 years for Kitui and Baringo samples respectively at 18±4 °C) at 18 ± 4°C but deteriorates fast when kept at higher temperatures. In some parts of Kenya like in North Eastern Province and parts of Coast Province where average daily temperatures of over 30°C are encountered, honey can only be stored for a maximum of 8 - 14 months, depending on the original level of its diastase activity. The same honey would store for up to 5 years in the cooler areas of the country such as Central Province and parts of the Rift Valley Province, where the average daily temperatures are about 14°C.

Based on the maximum storage of 2 years for honey recommended by both the Ministries of Health and Livestock Development (1988) of Kenya, it can be observed that only honey stored at 27° and 26°C for Kitui and Baringo samples in this study respectively would store for this period.

4.3.3 Changes in Colour

The results of changes in colour expressed in terms of optical density at 440 nm during various storage temperatures and time are shown in Table 27 and Figure 7. Analysis of variance showed that heat treatment during processing, storage temperature and time all contribute significantly (P = 0.05) to change in optical density. The storage temperature had the highest effect, followed by storage time and then the heat processing. The rate of increase in optical density increased with increase in storage temperature, time and heat treatment. This is in accordance with

Table 27: Mean Optical Densities (absorbance) of Kitui and Baringo Honey During Storage

Storage Time (Months)	Storage Temp (°C)	Optical Density (A440nm) Kitui	Baringo
Initial		0.39	0.24
	Room (18±4)	0.40 ± 0.01	0.25 ± 0.04
2	22	0.41 ± 0.01	0.26 ± 0.00
	28	0.43 ± 0.01	0.27 ± 0.00
	35	0.45 ± 0.01	0.29 ± 0.00
	18	0.43 ± 0.01	0.27 ± 0.00
4	22	0.44 ± 0.01	0.30 ± 0.01
	28	0.47 ± 0.01	0.29 ± 0.01
	35	0.50 ± 0.01	0.32 ± 0.00
	18	0.45 ± 0.00	0.28 ± 0.01
6	22	0.46 ± 0.00	0.30 ± 0.01
	28	0.52 ± 0.01	0.30 ± 0.01
	35	0.54 ± 0.01	0.33 ± 0.01

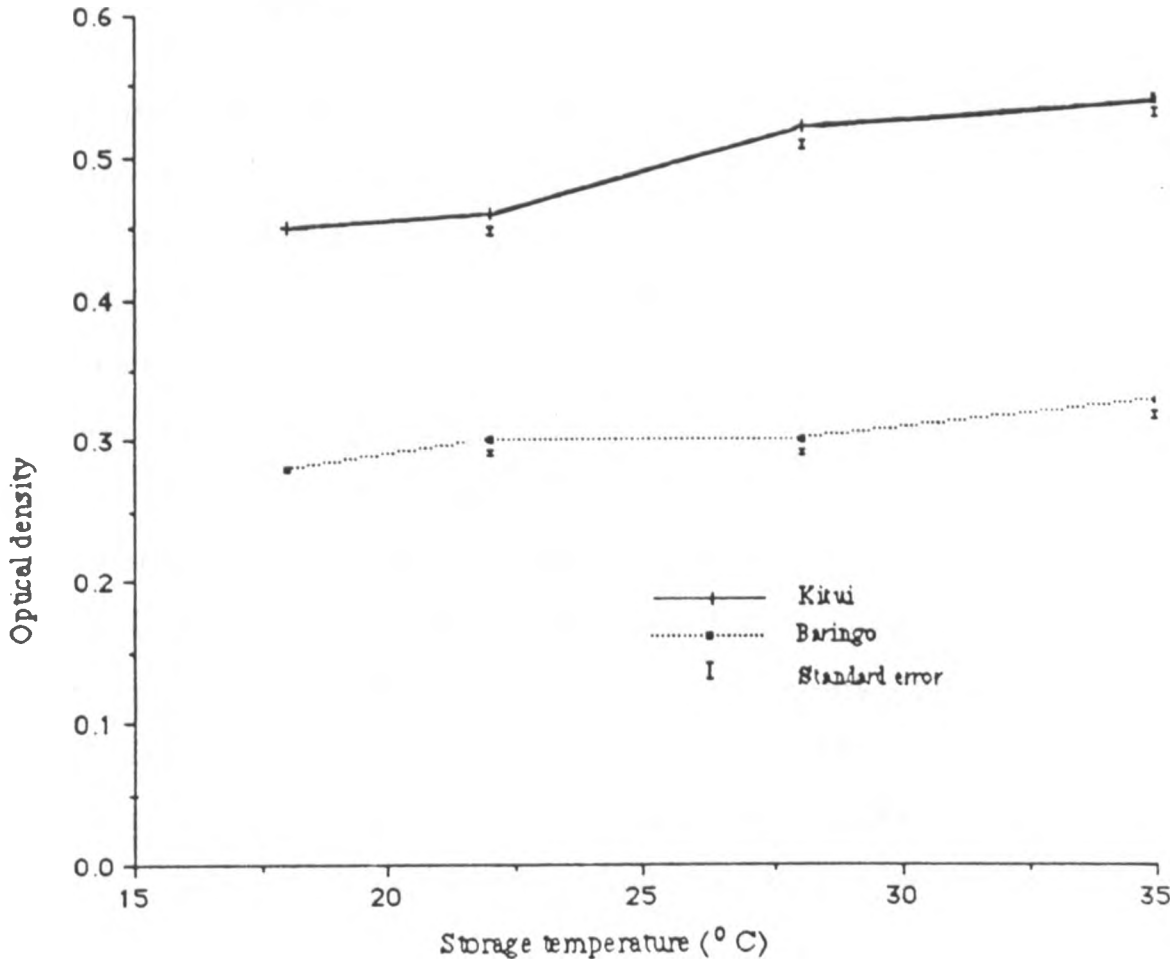

Figure 7: Effect of temperature (°C) on mean optical density (Absorbance at 440 nm) of processed Kitui and Baringo honeys after 6 months storage.

reports by Milum (1948). Of the two samples, however, the Kitui honey was more sensitive to heat than the Baringo honey as seen in Table 27 and Figure 7.

The optical densities for storage periods 0, 2, 4 and 6 months were all significantly different (P = 0.05) from each other.The effect of storage temperatures on the Kitui samples at 35°C was statistically different (P = 0.05) from the rest of the samples. The optical densities of the honeys at 28°C were also significantly different (P = 0.05) from optical densities of the honeys stored at 22° and 18°C whose values did not differ significantly from each other at the 5% level. For Baringo honey, storage at 35°C was, like for Kitui honey, significantly different from the rest of the three. The optical densities of the honeys stored at 18°C were found to differ significantly at 5% level from the optical densities of the honeys stored at 22° and 28°C) which did not differ significantly from each other at 5% level.

The optical densities of the Kitui unheated samples differed significantly (P = 0.05) from those of all the samples heated at at 45°, 50°, 55° and 60°C. However, values of all the four treatments were found not significantly different from each other. Similarly Baringo samples showed significant differences in optical densities at 5% level between the control and all the rest of the heat treated samples. There was also a significant difference between the optical densities of samples processed at 60°C and those at 45° and 50°C, which did not show significant

differences (P = 0.05) between from those heated at 55°C. These results do not agree with reports by White et al. (1964), who reported that dark honeys tended to be injured by heat at a higher rate than lighter honeys. In the present study all honeys were considered light.

All the samples showed changes in visual colour during storage as is shown in Table 28. From this Table it can be seen that storage at room temperature (18°±4°C) and at 22°C gave rise to products of the same colour in all cases, but they were significantly different (P = 0.05) in visual colour from those stored at 28° and 35°C. The samples stored at 28° and 35°C also differed significantly in colour from each other.

In this study, it was found that the rate of optical density increase was positively correlated to the rate of HMF production during storage. Since the optical density serves as an indicator of increased darkening, the present observations are in agreement with those of White et. al. (1964), who found that the rate of colour deterioration was related to HMF production.

The most interesting feature about the stored honey samples was the disappearance of the original hazy appearance, giving rise to transparency. This was accompanied by migration of the haze constituents to the top of the jars where they formed a semi solid, yellowish brown to a dark brown layer, the final colour depending on the origin of the sample, storage temperature and period. This physical phenomenon is shown in Plate 1.

Table 28: Colour[+] Changes in Kitui and Baringo Honey after Six Months' Storage at Different Temperatures

Storage Temp (°C)	Kitui 1		Kitui 2		Baringo 1		Baringo 2	
	Initial Colour	After 6 mths	Initial Colour	After 6 mths	Initial Colour	After 6 mths	Initial Colour	After 6 mths
Room 18±4°	Light amber	Amber	Amber	Amber	Extra white	Light amber	Light amber	Amber
22°	Light amber	Amber	Amber	Amber	Extra white	Light amber	Light amber	Amber
28°	Light amber	Dark amber	Amber	Dark amber	Extra white	Amber	Light amber	Dark amber
35°	Light amber	Dark	Amber	Dark	Extra white	Dark amber	Light amber	Dark

[+] The colour changes were determined by matching with pre-determined samples from the Beekeeing Branch of the Ministry of Livestock Development - Kenya

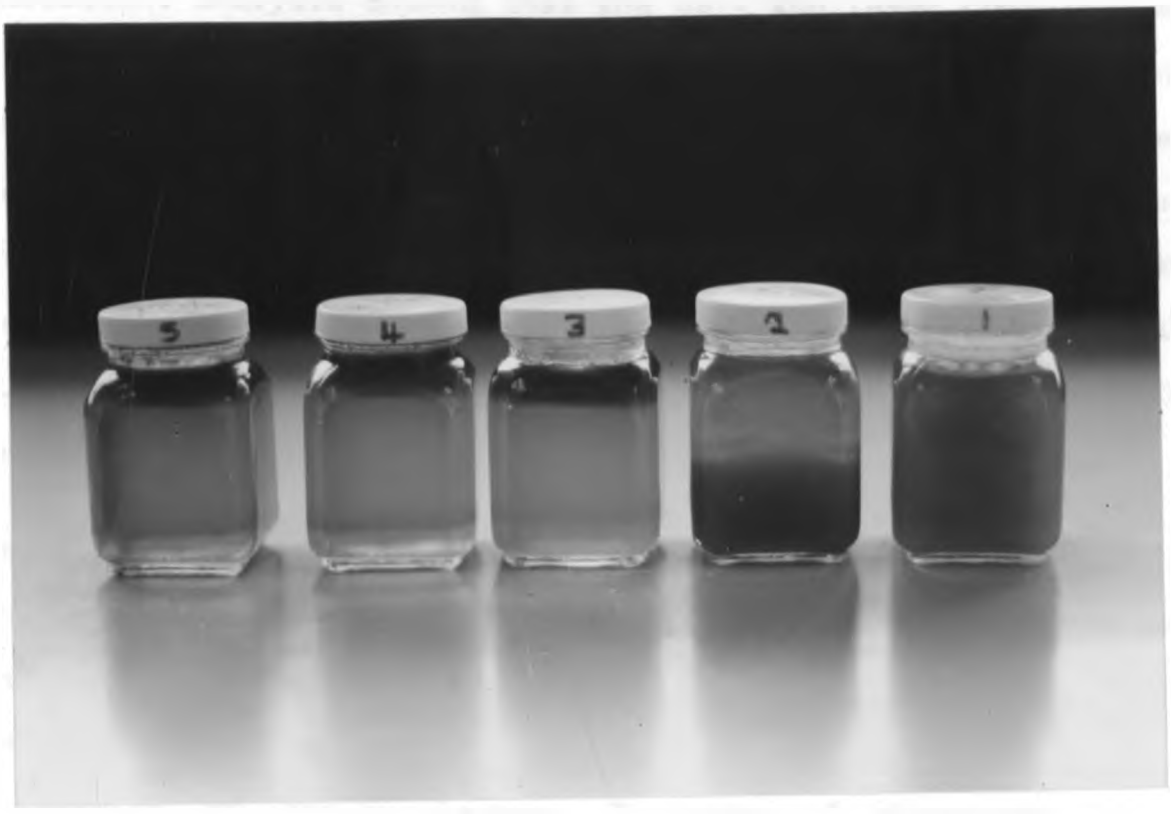

Plate 1: Effect of processing and storage temperatures on cloudness of honey. The honey samples had been stored for 2 months at 28°C. Note the pollen layer at the top at sample 5, 4 and 3. Also note fully crystalised and half crystallised samples 1 and 2 respectively.

Key

1 - Unheated honey sample (control)

2 - Sample heated at 45°C for 25 minutes

3 - Sample heated at 50°C for 25 minutes

4 - Sample heated at 55°C for 25 minutes

5 - Sample heated at 60°C for 25 minutes

Subsequent analysis showed that the dark top layer contained more nitrogen than the original samples and the transparent portion as shown in Table 29. It was therefore concluded that this layer consisted mainly of pollen, because it has been found that most of the nitrogenous material including proteins and amino acids come from pollen. The optical density of the top layer was also much higher than that of both the original honey and the transparent fluid portion (Table 29).

It was observed that the rate of the cloudness disappearance and the layer formation depended very much on the storage temperature and the degree of previous heat pre-treatment. The higher the pre-treatment amd storage temperatures, the faster was the separation of the two layers. The speed of migration of the pollen grains was assumed to be governed by Stokes law of viscosity thus:

$$V = \frac{D^2 g (e_p - e_f)}{18}$$

D = pollen grain diameter

g = gravitational acceleration

e_p, e_f = density of pollen grain and fluid respectively

V = viscosity of fluid (external phase)

The actual speed of separation at a given processing and storage temperatures as well as the forces governing the separation are subjects for further research.

Table 29: Nitrogen Contents and Optical Densities of the Pollen and the Serum Layers of Kitui and Baringo Honeys after Separation During Storage for 6 Months.

	Nitrogen %		Optical Density (A_{440})	
	Kitui	Baringo	Kitui	Baringo
Unstored, unseparated	0.5	0.4	0.314	0.203
Pollen layer stored	3.5	3.2	1.032	0.897
Serum layer stored	0.1	0.09	0.244	0.176

As already mentioned in 1.0, most Kenyan honeys look unattractive due to cloudness, which is associated with poor harvesting and such post harvest handling techniques as mashing of honey combs containing high levels of pollen in the process of which, a lot of pollen and other extraneous matter are incorporated resulting in the cloudy appearance.

4.3.4 Effect on Crystallization

Table 30 shows the effect of processing and storage temperatures on the days to first appearance of crystallization in four honey samples, two from each of Kitui and Baringo districts. Data show that there was decreased crystallization rate with increased processing temperatures at all storage temperatures for all samples.

From Table 30, it can also be seen that the rate of crystallization decreased with increased storage temperature. Virtually no crystallization occurred in the samples stored at 35°C even after 6 months. There was also very little crystallization in the samples stored at 28°C, which occured mainly in unheated samples and those heated at 45°C. These crystals were, however, easily redissolved with only little agitation of the jars.

Data on Table 28 also show that all samples stored at room (18±4°C) and 22°C did crystallize within 6 months, but at different rates depending on the level of previous heat treatment, the source of the sample and on the sample itself.

Table 30: Effect of Processing and Storage Conditions on the days to first Appearance of Crystallization in Kitui and Baringo Honeys

Storage Temp (°C)	Processing Temp (°C)	Days to First Appearance of Crystallization			
		Kitui 1	Kitui 2	Baringo 1	Baringo 2
Room (18±4°C)	Unheated	5	27	4	8
	45	11	49	16	18
	50	25	63	41	29
	55	50	74	72	53
	60	68	83	95	71
22°	Unheated	13	34	8	8
	45	19	83	36	26
	50	28	123	54	45
	55	75	*144	*79	62
	60	85	*163	*150	*83
28°	Unheated	*20	-	*72	*8
	45	*64	-	*118	-
	50	*84	-	-	-
	55	-	-	-	-
	60	-	-	-	-
35°	Unheated	-	-	-	-
	45	-	-	-	-
	50	-	-	-	-
	55	-	-	-	-
	60	-	-	-	-

* Crystallization present but crystals dissolved after shaking

The effect of processing temperature and sample source on rate of honey crystallization have already been explained in 4.2. It can be stated that the effect of storage temperature on rate of crystallization was in accordance with explanations of Lothrop (1943) and Kelly (1954).

It was observed that the unheated honeys crystallized to give fine crystals, and the crystallization process was complete within 8 days for Baringo 1 sample, but took 27 days for Kitui 2 sample, with the other two samples completely crystallizing for periods within this range. The heated honeys crystallized to give crystals that ranged from smooth and soft, almost creamy (Baringo 1 and 2); moderately smooth and hard (Kitui 1) to coarse grained (Kitui 2). Within a given sample, the crystals at lower heat treatments (45° and 50°C) were finer than those at higher heat treatments (55° and 60°C). In all cases the honeys heated at the temperatures of 55° and 60°C crystallized slowest. During storage at 28° and 35°C, no crystals had formed at all even after six months of storage. For honeys heated at 45° and 50°C, Baringo 2 and Kitui 1 showed complete crystallization by the end of six months storage period.

All the samples which had shown partial crystallization developed a foamy effervescence at the surface of the liquid layer. This was probably due to fermentation by the osmophilic yeast as a result of increased water activity in this area as explained in 2.6.6. This indicates that these products were of lower quality compared to those which had crystallized fully.

5. CONCLUSION AND RECOMMENDATIONS

Based on the results of the present study, high processing temperatures (55° and 60°C) result in increased delay in crystallization of honey. However, these high temperatures result in increased production of HMF, inactivation of honey enzymes and colour darkening all of which are indicators of low quality honey. This is especially crucial when the initial HMF values are high or the diastase activities are low, because such honeys will not keep for long during storage at high temperatures before exceeding the recommeded limits for these parameters. Lower processing temperatures will give rise to high quality products in terms of HMF generation and diastase inactivation as well as colour changes. However, such temperatures lead to high viscosity products which are difficult to strain resulting in high economic losses in terms of product and time. This also gives rise to fast crystallizing products. The most appropriate processing temperature is that which will minimize quality losses and delay crystallization at the same time. From the results of this study, processing at 55°C for 25 and 30 minutes result in a high quality product of a shelf-life beyond 6 months at 28° and 35°C. Processing at 55°C for 25 - 30 min is therefore recommended as the most appropriate processing conditions.

High storage temperatures of 28° and 35°C though retaining honey in liquid form for long periods resulted in products of low quality in terms of HMF, diastase level and colour indices.

Lower storage temperatures (18° and 22°C) maintained high quality of the honeys on the basis of the above parameters, but the onset of crystallization was early. In order to achieve the present storage requirement of 2 years storage, 25 - 26°C is recommended based on the present study. Within this temperature range honey can retain its quality for 2 years as measured in terms of HMF and diastase levels, and at the same time delay crystallization for long enough for the product to pass through the market. Initial bulk storage of honey at these temperatures can also be used to separate the pollen particles and other extroneous substances which impart cloudness to honey thereby improving on the general appearance and attractiveness.

There were significant differences among some parameters of samples from the two regions and also among samples of the same region collected at different times. This indicates that there is probably a great variation in honeys of this country due to the diversity of ecological zones. These variations which are both chemical and physical affect processing and storage requirements. There is need therefore for more research in honeys from different parts of the country to estimate the range of these chemical and physical characteristics for better processing and storage conditions recommendations.

This research could include the following:

(i) More assays of moisture, glucose, sucrose fructose, HMF and diastase activity in fresh honeys from different ecological zones, and determination of the appropriate processing and storage conditions.

(ii) Determination of the forces behind the pollen particles migration to the surface of the honey during storage with a view to quickening the separation to improve the quality as regards transparency and delayed crystallizzation.

(iii) Assay of organoleptic changes of honey after processing and during storage under predetermined conditions.

(iv) Test market survey for creamed honey in Kenya, which has a high potential for fast crystallizing honeys.

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Appendix I(a): Some important bee plant families and genera found in Kenya

PLANT FAMILY	COMMON GENERA
Acanthaceae	Ruellia, Justicia, Hypoestes, Asystacia, Barleria, etc.
Amaranthaceae	Amaranthus, Cyathula.
Boraginaceae	Cordia.
Capparidaceae	Ritchiae, Capparis.
Compositae	Vernonia, Aspilia, Senecio, Sonchus, Tarchonanthus.
Combretaceae	Combretum, Terminalia.
Cycadaceae	Encephalartos.
Euphorbiaceae	Croton, Drypetes, Euphorbia Acalypha, Bridelia.
Icacinaceae	Apodytes.
Labiatae	Ajuga, Becium, Endostemon, Leonotis, Leucas, Mentha, Ocimum, Salvia, Plectranthus.
Leguminosae	Acacia, Albizia, Bauhinia Cassia, Crotalaria, Cordyla, Indigotera, Craibia, Dalberia, Desmodium, Nowtonia, Afzelia, Julbernardia, Trifolium.
Malvaceae	Abutilon, Hibiscus Pavonia Sida, Urena
Melastomaceae	Memecylon, Dissotis
Myrtaceae	Eucalyptus, Callistemon.
Musaceae	Musa.
Rubbiaceae	Coffea, Mussaenda.

Appendix I (a) Cont'd

PLANT FAMILY	COMMON GENERA
Rutaceae	Citrus, Calodendrum, Teclea.
Sterculiaceae	Dombyea.
Thunbergiaceae	Thunbergia
Tiliaceae	Grewia
Umbelliferae	Heteromorpha, Sanicula.
Verbenaceae	Lippia, Clerodendrum, Vitex.

Source: Crane, 1975; Kahenya et al., 1985; Ngethe, 1985.

Appendix I(b): Common Acacia Species in Kenya

Acacia albida Del	–	Tree (up to 18 m); flowers early in wet season.
A. brevispica Harms	–	Shrub < 6 m, widespread at altitudes upto 2250 m.
A. drepanolobium Harms	–	Shrub of black cotton soil, < 4.5 m.
A. hockii De Wild	–	Grassland tree or shrub, < 4.5 m, common up to 2100 m.
A. Mellifera (vahl) Benth	–	Shrub or tree, < 7.5 m, flowers just before rains.
A. gerrardiie Benth	–	Grassland tree, < 12 m, common between 1200 and 2000 m
A. nitolica (L) Del	–	Grassland tree, producers numerous flowers early in rainy season.
A. nubica Benth	–	Open grassland shrub with numerous flowers.
A. polyacantha Wild	–	Fast growing, very localised tree with numerous flowers.
A. senegal (L) Wild	–	Widespread tree or shrub, flowers early in season.
A. seyal Del	–	Widespread clumped savannah tree, up to 9 m.
A. tortilis (Forssk) Hyane	–	Umbrella-shaped phreatophyte which produce numerous flowers during long rains.
A. xanthophloea	–	a riparian tree occuring all over Kenya

Source: Ngethe, 1985.

Appendix II: Pfund scale for different honey colours from Australia, Canadian and USA standards

Colour Description	Australia	Canada	U.S.A
Water white	-	-	8 mm
Extra white	17 mm	13 mm	17 mm
White	34 mm	30 mm	34 mm
Extra light amber	50 mm	-	50 mm
Golden	-	50 mm	-
Light amber	65 mm	85 mm	85 mm
Amber	90 mm	-	114 mm
Dark amber	114 mm	114 mm	Over 114 mm
Dark	-	Over 114 mm	-

Source: Crane, 1980

Appendix III(a): Physico-Chemical Composition of Baringo Honey after Processing at Various Temperatures (°C)$^{+}$ and Holding Time (Mins)$^{-}$

		45^{+}/20^{-}	45^{+}/25^{-}	45^{+}/30^{-}	50^{+}/20^{-}	50^{+}/25^{-}	50^{+}/30^{-}	55^{+}/20^{-}	55^{+}/25^{-}	55^{+}/30^{-}	60^{+}/20^{-}	60^{+}/25^{-}	60^{+}/30^{-}
	B_1	17.1	17.1	17.1	16.9	16.9	16.8	16.8	16.8	16.7	16.7	16.6	16.6
Moisture %	B_2	17.4	17.4	17.4	17.2	17.2	17.2	17.15	17.15	17.05	17.05	17.05	17.05
	B_1	78.8	78.5	77.8	77.8	77.3	78.8	77.5	77.3	77.1	76.9	77.0	76.8
Total Red Sugars %	B_2	74.0	74.1	73.8	73.1	74.0	73.7	73.1	73.0	72.8	73.2	63.4	72.3
	B_1	2.1	2.3	2.2	2.6	2.0	2.2	2.5	2.4	2.4	2.1	2.0	2.3
Sucrose %	B_2	3.0	3.1	2.8	2.9	2.6	2.6	2.9	3.0	2.8	3.0	2.6	2.9
	B_1	39.0	39.2	38.9	38.0	38.1	38.9	39.00	38.7	38.4	39.9	30.0	38.1
Glucose %	B_2	34.8	34.6	34.0	34.7	34.0	34.5	34.8	34.8	34.1	34.9	34.0	33.9
	B_1	43	42.5	42.1	43.0	42.4	43.1	41.6	41.7	41.8	40.0	42.2	41.8
Fructoase %	B_2	42.4	42.7	43.0	41.5	43.2	42.8	41.7	41.5	41.8	41.6	42.3	41.5
	B_1	30.4	30.6	29.9	31.0	28.1	30.5	30.9	31.2	29.0	31.0	30.1	30.9
Total Acid Meq/kg	B_2	24.6	25.4	24.9	24.0	27.1	24.5	24.5	24.6	26.0	24.0	25.1	24.1
	B_1	4.0	4.0	4.2	4.1	4.0	4.0	4.0	4.0	4.0	4.0	3.9	4.0
pH	B_2	3.8	3.8	3.8	3.8	3.8	3.9	3.8	3.8	3.8	3.8	3.9	3.8
	B_1	9.408	10.752	10.752	11.018	10.752	11.003	11.032	11.428	12.822	12.034	12.002	12.000
HMF Mg/kg	B_2	12.104	11.560	11.520	13.848	13.864	14.936	18.432	17.664	17.856	17.016	18.816	17.856
	B_1	19.18	19.10	19.14	19.08	19.14	19.09	19.16	19.05	19.13	19.06	19.10	19.08
Diastase value	B_2	15.06	15.06	15.01	15.02	15.08	15.00	15.10	15.01	15.01	14.97	15.00	15.00
Total water in soluble solids %	B_1	0.270	0.220	0.280	0.270	0.285	0.271	0.287	0.248	0.244	0.242	0.244	0.286
	B_2	0.110	0.110	0.107	0.110	0.115	0.109	0.113	0.112	0.116	0.118	0.116	0.114

Appendix III (a) Cont'd

		45°/20'	45°/25'	45°/30'	50°/20'	50°/25'	50°/30'	55°/20'	55°/25'	55°/30'	60°/20'	60°/25'	60°/30'
	B_1	0.29	0.27	0.28	0.32	0.31	0.29	0.30	0.32	0.35	0.29	0.26	0.26
Ash %	B_2	0.28	0.29	0.29	0.28	0.22	0.29	0.30	0.28	0.28	0.31	0.32	0.34
	B_1	0.250	0.250	0.250	0.252	0.251	0.253	0.251	0.253	0.252	0.252	0.253	0.254
Colour-Optical Density	B_2	0.202	0.208	0.208	0.203	0.207	0.234	0.250	0.244	0.244	0.207	0.238	0.254
	B_1	26.4	24.7	24.2	22.4	22.2	22.0	17.4	16.5	14.3	13.2	13.1	12.9
Viscosity Poises	B_2	23.1	17.6	16.5	13.2	12.1	12.1	11.0	11.0	11.0	9.9	9.35	8.8
	B_1	0.49	0.48	0.49	0.47	0.50	0.52	0.46	0.48	0.46	0.49	0.51	0.47
Nitrogen %	B_2	0.53	0.50	0.52	0.54	0.50	0.52	0.54	0.53	0.52	0.55	0.54	0.55

B_1 = Baringo sample 1

B_2 = Baringo sample 2

° = Processing temperature (RLC)

' = Holding time (Minutes)

Appendix III (b): Physico-Chemical Composition of Kitui Honey after Processing at Various Temperatures (°C)$^{+}$ and Holding Times (Mins)$^{-}$

		45^{+}/20^{-}	45^{+}/25^{-}	45^{+}/30^{-}	50^{+}/20^{-}	50^{+}/25^{-}	50^{+}/30^{-}	55^{+}/20^{-}	55^{+}/25^{-}	55^{+}/30^{-}	60^{+}/20^{-}	60^{+}/25^{-}	60^{+}/30^{-}
	K_1	18.6	18.6	18.6	18.5	18.5	18.5	18.4	18.4	18.4	18.3	18.3	18.3
Moisture %	K_2	19.1	19.1	19.1	19.0	18.9	18.9	18.85	18.8	18.7	18.6	18.5	18.5
	K_1	76.4	76.2	77.6	76.7	75.8	74.6	75.5	75.8	74.6	74.6	71.7	74.6
Total Red Sugars %	K_2	74.0	74.2	74.4	73.9	73.0	73.0	73.1	73.4	73.6	73.6	72.5	73.8
	K_1	3.6	3.7	3.5	3.1	3.3	3.3	3.4	4.2	3.6	3.9	3.8	3.9
Sucrose %	K_2	1.5	1.4	1.6	1.2	1.5	1.6	1.4	1.2	1.3	1.2	1.1	1.3
	K_1	37.1	37.1	37.2	36.9	36.7	37.0	37.1	36.9	37.1	36.9	37.0	36.9
Glucose %	K_2	36.1	36.3	36.0	35.7	35.4	36.0	35.4	35.4	35.3	36.0	36.0	35.9
	K_1	42.5	42.3	42.7	43.0	42.3	40.6	41.5	42.1	40.5	40.8	37.5	40.8
Fructoase %	K_2	41.4	41.0	41.5	41.3	40.6	40.0	40.7	40.0	41.4	40.6	39.5	40.1
	K_1	29.5	30.6	29.3	29.9	29.0	30.9	29.4	31.0	29.0	30.3	30.4	29.4
Total Acid Meg/kg	K_2	27.1	26.4	26.7	26.3	27.8	25.7	26.2	25.2	27.6	26.7	26.3	27.7
	K_1	3.6	3.55	3.6	3.6	3.6	3.55	3.6	3.55	3.6	3.6	3.55	3.6
pH	K_2	4.2	4.25	4.2	4.2	4.2	4.25	4.2	4.25	4.2	4.2	4.25	4.2
	K_1	12.54	12.547	13.064	13.441	13.443	13.627	13.822	13.820	13.634	13.981	13.981	14.036
HMF Mg/kg	K_2	11.228	10.698	11.214	11.280	12.414	13.218	14.666	14.664	14.806	15.366	15.902	15.074
	K_1	21.40	21.43	21.48	21.38	21.40	21.40	21.36	21.38	21.42	21.38	21.33	21.34
Diastase value	K_2	18.61	18.84	17.79	17.45	18.04	17.94	17.70	17.70	17.43	18.19	18.05	17.27
Total water in soluble solids %	K_1	0.109	0.107	0.109	0.104	0.110	0.113	0.109	0.111	0.114	0.108	0.113	0.110

Appendix III (b) Cont'd

		45°/20⁻	45°/25⁻	45°/30⁻	50°/20⁻	50°/25⁻	50°/30⁻	55°/20⁻	55°/25⁻	55°/30⁻	60°/20⁻	60°/25⁻	60°/30⁻
	K_1	0.33	0.30	0.28	0.32	0.33	0.34	0.29	0.31	0.30	0.30	0.33	0.34
Ash %	K_2	0.39	0.44	0.46	0.40	0.43	0.40	0.47	0.47	0.44	0.44	0.41	0.40
	K_1	0.363	0.365	0.365	0.375	0.377	0.376	0.378	0.381	0.381	0.379	0.380	0.382
Colour-Optical Density	K_2	0.408	0.398	0.406	0.410	0.411	0.416	0.414	0.414	0.418	0.416	0.420	0.429
	K_1	13.8	11.0	10.8	9.7	9.2	8.8	7.7	7.7	7.4	6.9	6.6	6.5
Viscosity Poises	K_2	9.3	9.3	9.2	8.9	8.6	8.8	8.1	8.1	7.4	7.3	7.1	7.0
	K_1	0.47	0.40	0.44	0.43	0.45	0.40	0.39	0.42	0.43	0.42	0.44	0.42
Nitrogen %	K_2	0.36	0.37	0.35	0.36	0.36	0.37	0.35	0.39	0.36	0.37	0.36	0.38

K_1 = Kitui sample 1

K_2 = Kitui sample 2

Appendix III (c): Mean (two replications) Hydroxymethylfurfural Content (mg/kg) of Processed Kitui and Baringo Honeys during Storage at different conditions

Storage Time (Months)	Storage Temperature (°C)		PROCESSING TEMPERATUTE (°C)* AND TIME (Mins)~												
			Unheated	45*/20~	45*/25~	45*/30~	50*/20~	50*/25~	50*/30~	55*/20~	55*/25~	55*/30~	60*/20~	60*/25~	60*/30~
0	Room (18*4)	Kitui	5.14	11.88	11.62	12.14	12.36	12.93	13.42	14.24	14.24	14.22	14.67	14.91	14.55
		Baringo	7.72	9.98	11.13	11.99	11.94	11.62	13.47	14.73	14.54	14.84	13.03	15.41	14.93
2	Room	Kitui	5.49	14.04	13.83	13.59	14.19	13.85	14.84	15.02	15.0	15.18	15.68	15.60	15.17
		Baringo	8.32	11.71	12.70	12.76	14.50	12.61	14.98	15.71	15.88	15.84	14.97	16.16	16.15
	22	Kitui	6.81	15.32	15.32	15.50	16.10	15.92	15.71	16.17	16.10	16.53	16.21	16.24	16.90
		Baringo	9.28	12.53	13.54	15.00	15.17	14.52	16.46	17.05	17.33	17.31	15.77	17.50	17.68
	28	Kitui	11.72	15.35	17.94	16.67	16.16	17.75	18.15	18.68	19.57	19.16	19.65	19.67	20.92
		Baringo	17.54	19.89	21.12	22.12	22.92	24.82	25.91	27.44	24.64	25.11	25.52	25.46	25.75
	35	Kitui	23.22	33.30	37.71	32.72	31.85	33.27	31.74	36.74	29.34	32 81	33.10	34.33	35.29
		Baringo	56.21	59.38	64.37	57.22	60.10	63.69	77.71	79.38	71.71	73.27	82.76	83.63	84.14
4	Room	Kitui	5.57	14.30	14.50	14.59	14.87	15.50	15.50	15.93	16.06	16.39	17.91	18.67	19.00
		Baringo	9.12	12.48	13.11	14.11	15.94	14.21	16.13	16.51	17.57	17.70	15.55	17.40	17.38
	22	Kitui	7.45	16.42	16.80	17.05	17.22	17.54	17.90	18.62	18.71	18.68	18.71	19.26	19.52
		Baringo	10.34	13.34	14.78	14.88	16.03	19.01	18.14	18.53	20.64	19.41	17.47	20.16	19.39
	28	Kitui	14.73	18.42	20.81	21.01	25.53	25.43	26.86	24.46	23.50	25.42	25.71	26.10	26.62
		Baringo	27.65	29.86	31.19	32.06	35.12	35.44	36.90	38.15	35.14	36.00	35.64	38.11	37.08
	35	Kitui	63.30	69.73	71.74	72.99	72.12	72.51	76.06	78.81	76.96	73.31	74.74	77.68	78.75
		Baringo	113.79	120.96	125.76	121.26	126.16	127.98	145.21	146.78	144.39	151.07	155.12	153.39	157.55

Appendix III (c) Cont'd

Storage Time (Months)	Storage Temperature (°C)		PROCESSING TEMPERATUTE (°C)$^{\circ}$ AND TIME (Mins)$^{-}$												
			Unheated	45°/20-	45°/25-	45°/30-	50°/20-	50°/25-	50°/30-	55°/20-	55°/25-	55°/30-	60°/20-	60°/25-	60°/30-
6	Room	Kitui	6.07	14.98	15.02	15.20	15.72	16.06	16.03	16.92	17.38	17.38	18.82	19.80	20.92
		Baringo	10.44	14.79	15.36	15.74	16.32	16.70	18.14	18.05	18.01	18.05	17.47	19.01	18.34
	22	Kitui	9.60	17.86	18.80	19.78	18.43	19.90	20.33	20.83	20.44	21.10	22.85	22.88	23.52
		Baringo	13.80	16.42	16.32	17.95	16.51	17.66	20.06	20.64	21.02	20.54	20.54	20.93	22.08
	28	Kitui	27.84	32.42	37.81	40.21	41.54	40.59	43.19	42.90	42.98	43.70	42.30	42.01	44.97
		Baringo	37.73	44.45	41.83	43.68	43.78	45.79	46.27	47.06	41.29	46.66	46.48	48.48	48.86
	35	Kitui	190.46	206.99	211.08	216.98	218.19	221.87	221.20	222.55	222.79	228.83	231.06	222.53	231.08
		Baringo	220.95	234.48	245.86	255.12	243.21	251.05	270.67	270.77	274.18	272.26	284.79	286.07	284.00

Appendix III (d): Mean (two replications) Optical Density [illegible] of Processed Kitui and Baringo Honeys during Storage at different conditions

Storage Time (Months)	Storage Temperature (°C)		PROCESSING TEMPERATURE (°C)* AND TIME (Mins)~												
			Unheated	45*/20~	45*/25~	45*/30~	50*/20~	50*/25~	50*/30~	55*/20~	55*/25~	55*/30~	60*/20~	60*/25~	60*/30~
0	Room (18-24)	Kitui	0.314	0.386	0.382	0.386	0.393	0.392	0.396	0.396	0.398	0.400	0.398	0.400	0.410
		Baringo	0.203	0.226	0.229	0.229	0.228	0.229	0.244	0.251	0.249	0.248	0.230	0.246	0.254
60	Room	Kitui	0.326	0.404	0.397	0.394	0.396	0.397	0.400	0.404	0.406	0.405	0.403	0.407	0.421
		Baringo	0.212	0.239	0.245	0.250	0.248	0.242	0.257	0.264	0.262	0.264	0.260	0.265	0.269
	22	Kitui	0.366	0.416	0.404	0.415	0.417	0.422	0.416	0.416	0.419	0.418	0.426	0.426	0.434
		Baringo	0.234	0.244	0.252	0.256	0.254	0.260	0.269	0.274	0.272	0.274	0.257	0.276	0.279
	28	Kitui	0.373	0.424	0.415	0.427	0.418	0.425	0.432	0.435	0.444	0.435	0.435	0.438	0.438
		Baringo	0.246	0.257	0.266	0.266	0.270	0.278	0.281	0.284	0.281	0.273	0.271	0.285	0.289
	35	Kitui	0.387	0.442	0.439	0.443	0.449	0.447	0.446	0.485	0.454	0.449	0.447	0.453	0.458
		Baringo	0.267	0.279	0.282	0.279	0.272	0.289	0.303	0.304	0.294	0.295	0.291	0.311	0.309
120	Room	Kitui	0.353	0.427	0.419	0.430	0.428	0.432	0.436	0.444	0.432	0.440	0.439	0.438	0.441
		Baringo	0.222	0.255	0.261	0.269	0.263	0.260	0.276	0.285	0.279	0.281	0.276	0.273	0.286
	22	Kitui	0.388	0.437	0.441	0.439	0.436	0.442	0.443	0.452	0.445	0.454	0.453	0.452	0.454
		Baringo	0.261	0.280	0.290	0.299	0.292	0.292	0.301	0.304	0.296	0.299	0.281	0.283	0.296
	28	Kitui	0.416	0.448	0.476	0.477	0.475	0.479	0.482	0.466	0.480	0.482	0.486	0.485	0.489
		Baringo	0.262	0.282	0.297	0.297	0.300	0.303	0.302	0.308	0.305	0.306	0.291	0.309	0.307
	35	Kitui	0.454	0.485	0.493	0.497	0.494	0.496	0.506	0.505	0.508	0.514	0.516	0.516	0.519
		Baringo	0.278	0.309	0.313	0.310	0.319	0.321	0.328	0.328	0.333	0.328	0.330	0.335	0.339

Table III (d) Cont'd

Storage Time (Months)	Storage Temperature (°C)		PROCESSING TEMPERATURE (°C)$^{\circ}$ AND TIME (Mins)$^{-}$												
			Unheated	45°/20⁻	45°/25⁻	45°/30⁻	50°/20⁻	50°/25⁻	50°/30⁻	55°/20⁻	55°/25⁻	55°/30⁻	60°/20⁻	60°/25⁻	60°/30⁻
180	Room	Kitui	0.369	0.452	0.452	0.443	0.455	0.455	0.457	0.458	0.459	0.458	0.460	0.462	0.465
		Baringo	0.237	0.274	0.271	0.276	0.272	0.267	0.275	0.302	0.302	0.299	0.289	0.288	0.300
	22	Kitui	0.398	0.445	0.456	0.457	0.459	0.455	0.462	0.464	0.463	0.466	0.471	0.468	0.473
		Baringo	0.259	0.288	0.293	0.304	0.293	0.300	0.303	0.304	0.300	0.302	0.306	0.294	0.298
	28	Kitui	0.442	0.511	0.519	0.518	0.524	0.525	0.522	0.526	0.526	0.529	0.529	0.531	0.531
		Baringo	0.268	0.298	0.306	0.306	0.305	0.309	0.309	0.309	0.314	0.311	0.305	0.323	0.319
	35	Kitui	0.504	0.527	0.536	0.539	0.541	0.541	0.546	0.543	0.545	0.552	0.554	0.555	0.555
		Baringo	0.289	0.321	0.324	0.325	0.328	0.330	0.337	0.340	0.337	0.337	0.339	0.343	0.345

Appendix III (a) Mean (two replications) Diastase number of Processed Kitui and Baringo Honeys during Storage at different conditions

Storage Time (Months)	Storage Temperature (°C)		PRE-TREATMENT DIASTASE (°C)$^{+}$ AND TIME (Mins)$^{-}$												
			Unheated	45^{+}/20^{-}	45^{+}/25^{-}	45^{+}/30^{-}	50^{+}/20^{-}	50^{+}/25^{-}	50^{+}/30^{-}	55^{+}/20^{-}	55^{+}/25^{-}	55^{+}/30^{-}	60^{+}/20^{-}	60^{+}/25^{-}	60^{+}/30^{-}
0	Room (18°4)	Kitui	20.09	20.01	20.14	19.64	19.41	19.72	19.67	19.53	19.54	19.43	19.79	19.69	19.31
		Baringo	17.72	17.07	17.08	17.07	17.10	17.11	17.05	17.18	17.03	17.07	17.02	17.05	17.05
60	Room	Kitui	19.94	19.65	19.75	19.25	19.03	19.34	19.25	19.18	19.23	19.06	19.45	19.31	19.14
		Baringo	16.94	16.85	17.03	16.90	16.69	16.85	16.92	16.86	16.63	16.78	16.70	16.71	16.97
	22	Kitui	19.68	19.61	19.07	18.62	18.32	18.78	18.60	18.68	18.91	18.78	18.73	18.80	18.50
		Baringo	16.80	16.75	16.91	16.79	16.65	16.53	16.69	16.68	16.55	16.57	16.51	16.57	16.55
	28	Kitui	18.25	17.97	17.96	18.01	17.83	18.01	18.00	17.86	17.96	17.99	17.92	17.88	17.91
		Baringo	15.29	14.80	16.13	14.43	14.54	14.11	13.95	14.49	14.22	14.42	14.40	14.71	14.29
	35	Kitui	12.57	12.45	12.28	12.68	11.95	12.25	21.10	12.24	12.34	12.61	11.84	11.97	12.15
		Baringo	11.03	10.76	10.03	10.31	10.06	10.54	10.20	10.45	10.79	10.47	10.28	10.04	10.24
120	Room	Kitui	19.38	19.39	19.32	18.56	18.83	19.02	18.58	18.87	18.48	18.68	18.92	18.76	18.31
		Baringo	16.72	17.43	16.50	16.20	15.79	16.29	16.32	16.11	16.08	16.22	16.05	16.07	16.01
	22	Kitui	19.04	19.18	18.02	18.1	17.96	18.28	18.19	18.24	17.96	18.35	18.04	17.83	17.88
		Baringo	16.25	16.07	15.69	15.81	16.05	15.97	15.70	15.97	16.00	15.95	15.87	15.88	15.73
	28	Kitui	16.22	16.01	16.07	16.12	16.14	16.12	16.03	15.56	16.03	15.84	15.56	16.11	15.81
		Baringo	13.46	13.22	12.83	12.74	13.08	12.72	12.66	12.54	12.58	12.75	12.40	12.59	12.60
	35	Kitui	6.44	6.26	6.26	5.98	5.98	5.86	5.90	6.13	6.22	5.77	6.31	5.82	5.79
		Baringo	6.90	6.79	6.57	6.42	6.90	6.34	6.55	6.24	6.54	6.54	6.23	6.59	6.58

Appendix III (e) Cont'd

Storage Time (Months)	Storage Temperature (°C)		PROCESSING TEMPERATUTE (°C)$^{\phi}$ AND TIME (Mins)$^{-}$												
			Unheated	45$^{\phi}$/20^{-}	45$^{\phi}$/25^{-}	45$^{\phi}$/30^{-}	50$^{\phi}$/20^{-}	50$^{\phi}$/25^{-}	50$^{\phi}$/30^{-}	55$^{\phi}$/20^{-}	55$^{\phi}$/25^{-}	55$^{\phi}$/30^{-}	60$^{\phi}$/20^{-}	60$^{\phi}$/25^{-}	60$^{\phi}$/30^{-}
180	Room	Kitui	18.54	18.96	18.54	18.14	18.46	18.57	17.98	18.40	17.98	18.70	18.41	17.90	17.75
		Baringo	16.06	16.00	15.83	15.60	15.67	15.67	15.64	15.95	15.68	15.57	15.56	15.63	15.86
	22	Kitui	18.45	18.25	17.29	16.80	17.29	17.06	16.87	17.02	16.74	17.50	17.48	17.02	17.12
		Baringo	15.57	15.56	15.25	15.42	15.75	15.42	15.56	15.27	15.21	15.43	15.24	15.31	15.51
	28	Kitui	14.25	14.19	14.01	14.12	14.35	14.06	13.84	14.04	13.94	13.77	14.03	13.88	13.88
		Baringo	10.98	10.61	10.42	10.43	10.28	10.71	10.46	10.39	10.81	10.32	10.13	10.32	10.66
	35	Kitui	4.35	4.11	4.01	3.99	4.31	4.28	3.86	3.59	4.35	4.39	3.61	3.59	3.87
		Baringo	4.97	4.94	4.77	4.88	5.09	4.51	4.51	4.66	4.80	4.86	4.35	4.50	4.47