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Factors influencing quality of palm oil produced at the cottage industry level in Ghana

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Abstract: Palm oil is one of the major fats and oils produced and traded in the world today. It forms an important ingredient in the diet of many people in West Africa. The quality of palm oil is mostly determined by the fatty acid, moisture and impurity content, and bleachability. This study examined the quality of palm oil produced at cottage industry level in Ghana with a view to improve its quality to meet industrial standards and increase its marketability. Palm oil produced from fruits stored at various periods with various levels of contamination were examined over a twelve-month storage period for their microbial, fatty acid, moisture and impurity contents. PCR amplifications, sequencing and fragment analysis methods were used to identify microbes, while fatty acid (FFA), moisture and impurity indices were determined by titration and oven-dry methods. The major factors determining the fatty acid content was age of fruits used to process the oil and the length of storage of the oil after processing. The results show that good grade oils with low fatty acid (FFA) contain microbes, some of which have the potential of producing toxin. There was a direct relationship between storage period of oil and fatty acid content, and microbial loads in oils. As storage period increased, fatty acid and microbial load increased (r = 0.972 and 0.967). Lipolytic organisms identified are *Aspergillus* (0.27), *Bacillus* (0.08), *Candida* (1.34), *Geotrichum* (1.00) and *Pseudomonas* (2.11) species.

Keywords: Fatty acid, fragment analysis, Ghana, impurity, microbes, moisture, palm oil

Introduction

The oil palm ranks among the most important oil producing crops in Sub-saharan Africa, and is one of the most important sources of edible oil. It produces one of the major oils and fats traded on the continent and the world today. Palm and palm kernel oils contributed 36.1% of the world's total oils and fats in 2007/2008 (Mielke, 2008).

Palm oil is processed from the fresh fruits using various methods, and these techniques differ in the level of mechanisation and interconnecting material transfer mechanisms. The scale of operations also differs at the level of processing and this affects the quality of the final product. The operations can be grouped into four, namely traditional methods, small-scale mechanical units, medium-scale and large industrial mills. The small-scale mills generally process up to two tonnes fresh fruit bunch (FFB) per hour while the large-scale mills process from 10 to 60 tonnes FFB per hour (Poku, 2002).

Most of the palm oil produced in Ghana for human consumption is processed at the cottage industry (small-scale) level. Visual observation of the palm fruits used to process oil shows various levels of microbial contamination. The populations of these microbes increase over time as these fruits are stored in open piles prior to processing. Although there are reports on the thermophilic microflora of oils such as rapeseed oil and palm oil (El Azzabi *et al*., 2006; Oso, 2007; O-Thong *et al*., 2008; Ismail *et al*., 2010), most of the work done on oil quality is related to the free fatty acid and oil degradation. Investigations on the biodeterioration potentials of fungi isolated from vegetable oils shows that some fatty acid components of the oil were lost due to yeast growth in the oils while new ones were synthesised (Molokwu and Okpokwasili, 1997). Edible mushrooms (*Pleurotus* spp.) were also cultivated in olive oil mill waste sites for biological remediation (Zervakis *et al*., 1996). PCR amplifications and Terminal Restriction Fragment analysis methods were therefore used to identify microbes on the fruits and the oil. Terminal Restriction Fragment Length Polymorphism (TRFLP) have been used to monitor and describe populations of microbes in diverse environments such as soils (Ranjard *et al*., 2001; Blackwood *et al*., 2003), food and water (Randazzo *et al*., 2002; Ercolini *et al*., 2003; Pérez-Luz *et al*., 2004) and compost (Schloss *et al*., 2003).

Fats and oils are graded by their acid and free fatty acid (FFA) contents, which are used as an index to determine their quality (Kardash and Tur'yan, 2005). The major fatty acids predominant in palm oil are oleic and palmitic acids (Salunkhe *et al*., 1992). Fatty acids play a very important role in fats and oils

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because of their health implications in the human diet and properties in industrial processes. The type of fatty acid determines the nutritional status and storability (keeping quality) of the oil. Palmitic acid increases in palm oil are mostly associated with oils produced from over-ripe, bruised and crushed fruits, fruits subjected to severe impacts from loading and off-loading bunches and oils stored over long periods (Salunkhe *et al*., 1992; Tagoe, 2008). Autocatalytic hydrolysis of the oil in the presence of moisture also increases the fatty acid content (Purseglove, 1975). Free fatty acids in oils and fats may also be caused by the action of microbial lipases (Odunfa, 1989).

This study was conducted to identify microbes that occur on oil palm fruits and palm oil and assess their effect on the quality of oil produced at the cottage industry level.

Materials and Methods

Study site

The study was conducted at a private commercial mill at Kusi in the Eastern Region located in the Moist Semi-deciduous forest zone of Ghana. The site lies between latitude -N 06˚C, 01', 54.3'' and longitude -W 000˚C 52', 30.8''. The area has a mean annual water deficit of 150-250mm for oil palm cultivation. It therefore, falls within the optimum and favourable ecozones for oil palm cultivation. The bulk of the palm oil in Ghana is produced in this Region.

Processing and physico-chemical composition of palm fruits

Freshly harvested palm fruit bunches from the field were immediately chopped (cut) with an axe after harvesting. The fruits were picked from the spikelets and 0.5 tons loose fruits were stored in an open pile of 63.0 cm for 0, 3, 6, 9, 12, 19 and 26 days before processing (Fig 1). Temperature within the fruits and immediate environment, relative humidity of the area, pH of the fruit mesocarp and moisture content of the fruit mesocarp were measured. Temperature of fruits was taken at different slopes of the pile using a thermometer and the mean value determined. Relative humidity measurements for the area were provided by the Meteorological Services Department. pH of the fruit mesocarp was obtained by mashing the mesocarp. The mashed mesocarp was mixed with water in a ratio of 1:2.5 and pH values were determined using a Jenway 3305 pH meter. pH measurements were taken in four replicates and the mean value determined. Fruits from the various storage periods were processed into oil using a small-scale mechanical unit and the free fatty acid (FFA), moisture and impurity contents determined.

Figure 1. Levels of contamination of palm fruits stored for 26 days. (A) 0 days, fresh fruits (B) 3 days, (C) 6 days, (D) 9 days, (E) 12 days, (F) 19 days, (G) 19 days, (H) 26 days, (I) 26 days.

Microbial evaluation

Culture and non-culture dependent methods were used for microbial analysis and PCR amplifications and Terminal Restriction Fragment analysis methods were also used to identify microbes on the fruits and the oil.

DNA preparation from oil palm samples

Various DNA extraction methods (DNeasy kits, bead beating, NaOH buffers and GES extraction buffers) were used to isolate DNA that could be amplified by PCR with both fungal and bacterial ribosomal primers. Pellets and cultures were obtained by washing palm fruits in 50 mM Potassium Phosphate buffer and tween 80. The solutions were spinned at 10,000 x g for 5-10 minutes to obtain pellets for DNA preparation. A second half of the washing solution was serially diluted for culturing on Potato Dextrose Agar (PDA) and Czapek Dox Agar plates (with and without antibiotic amendments). The DNeasy plant mini and maxi kits (Qiagen, UK) were used for DNA preparation from pellets and cultures following the manufacturer's instructions. DNAs were eluted in 50-100 µl elution buffer. To extract from palm oil samples, 200µl palm oil was frozen and ground in liquid nitrogen. Lysis buffer ASL from the QIAamp stool kit (Qiagen, UK), and acid-washed glass beads were added and the samples were bead beaten as described above. Manufacturer's instructions were then followed to prepare the DNA which was eluted in 50 µl elution buffer.

PCR and TRFLP analyses

Amplification of fungal and bacterial DNA from fruit and oil samples were as described in Hodgetts

et al. (2007) and Yu *et al*. (2009). The fungal primers used were Fitsrev (5'-ATA TGC TTA AGT TCA GCG GGT-3') and 5.8S for (5'-TCG ATGAAG AAC GCA GG-3') (Yu *et al*., 2009). The bacterial primers used were 23Srev (5'-TTC GCC TTT CCC TCA CGG TAC T-3') and 23Sfor (5'- GCG ATT TCY GAA YGG GGR AAC CC -3') (Anthony *et al*., 2000). Five µl of PCR product was digested in 10 µl reaction volumes containing 1 U *Hae*III restriction enzyme (for fungal PCR products) or 1 U *Mse*I (for bacterial products), and incubated at 37°C for 2 hr to ensure complete digestion. Fragment analysis methods and analysis of data were performed as described in Hodgetts *et al*. (2007).

Sequencing from palm oil DNA

PCR products obtained from palm oil DNAs were cleaned using the Gen Elute™ PCR Clean-Up Kit (SIGMA) before sequencing. Sequences were processed on one strand only using the Beckman Quickstart kit technology which uses WellRed Dye chemistry (infra-red dyes), with a CEQ 8000 Genetic Analysis System and the resultant sequences were analysed with BLASTN through the National Centre for Biotechnology Information (NCBI) website (*http://www.ncb.nlm.gov/BLAST*, 2007), by aligning input sequences against published nucleotide sequences to determine the most likely identity of the bacteria and fungi.

Palm Oil Quality Analyses

Palm oil processed from fruits at various storage periods (0, 3, 6, 9, 12, 19, 26 days) and selected oil samples from the small scale mills were stored for one year. The aim was to study the rate of deterioration (storability) of the samples. The free fatty acid, moisture content and percentage impurity (dirt) content of the oils were determined. All samples were preheated to 50˚C before use. Methods for oil quality analyses were adopted from routine methods used by the Ghana Oil Palm Development Company Limited (GOPDC).

Free fatty acid (FFA) content

A clean dry beaker was weighed to the nearest 0.001 g (W1) and 2 g of pre-heated oil (heated to about 50°C) was added and reweighed (W2). Aliquots of ethanol (50 ml) was added to the oil to completely free the fatty acids and the ethanol-oil mixture was titrated with 0.1N NaOH using phenolphthalein indicator. The volume (V) of NaOH required to produce the first permanent pink colour was recorded and the free fatty acid content of the oil was determined from the formula:

$$\% \text{ FFA} = \frac{M \times V \times N}{10 \times m}$$

where:

M = Relative molecular mass of Palmitic acid = 256, V = volume of NaOH used, N = Normality (concentration) of NaOH used, m = Weight of oil used.

Moisture content

McCartney bottles were washed with water and oven dried for 30 minutes. They were weighed to the nearest 0.001 g (W1) and 2 g of oil added and reweighed (W2). The samples were dried at 105˚C for 4 hr and cooled in a desiccator and re-weighed (W3). All samples were replicated three times. Moisture content of the oils was determined by the formula below.

$$\% \text{ Moisture} = \frac{\text{W2-W3 (moisture loss)}}{\text{W2-W1 (fresh weight)}} \text{ X } 100$$

Where:

W1 = Weight of McCartney bottle, W2 = Weight of McCartney bottle + oil, W3 = Dry Weight of McCartney bottle + oil

Impurity content

Gooch crucibles were lined with filter paper, washed with hexane, and dried at 105˚C for 30 minutes. The crucibles were allowed to cool and weighed (W1). Erlenmeyer flasks (250 ml) were weighed (W2) and 2 g of oil added and reweighed (W3). Hexane (20 ml) was added to the oils and the flasks swirled and heated to homogenize the mixtures. The mixtures were poured into the crucibles and allowed to drain. The flasks were rinsed with hexane and poured into the crucibles. This was done to remove any particles present in the flasks. The crucibles were removed after all the solutions had drained and dried at 105˚C for 30 minutes. They were cooled and reweighed (W4). Impurity in the oil was expressed as a percentage from the formula below.

$$\% \text{ Impurity (Dirt)} = \frac{\text{W4-W1 (dry weight of oil)}}{\text{W3-W2 (fresh weight of oil)}} \text{ X } 100$$

Where:

W1 = Weight of crucible + filter paper, W2 = Weight of flask, W3 = Weight of flask + oil, W4 =Dry weight of crucible + oil

Statistical Analysis

Samples were analysed in three replicates and results subjected to statistical analysis using GenStat 10.1.

Results and Discussion

Physico-chemical composition of palm fruits

Temperature within the fruits increased with the age of fruits and decreased with size of the pile. Moisture content of the fruits however, decreased with age, while contamination and deterioration of the fruits increased with age. The longer the fruits were stored, the higher the contamination and deterioration (Figure 1) and the higher the free fatty acid content of the oil (Table 2).

Microbial evaluation

The only persistent organism in oil from the fresh fruits is *Hirsutella* spp. (Figure 2a). Occurrence of fungal species in oil from the 12-day storage fruits was highly variable (Figure 2c). Peak area ratio for the fungal spp increased with increase in storage period, i.e. the longer the storage period the higher the fungal population. *Hirsutella spp*. appears to be prominent in oils from fresh, 12 and 26 days fruits due to its higher peak area ratio and population (Figure 2). The peak area ratio for *Hirsutella* is 3.02 compared to 1.94 and 1.93 for Candida and Geotrichum respectively (Table 1A).

For the bacteria spp, peak area ratio of *Leuconostoc* and *Waddlia* spp. were variable (Figure 3). *Pseudomonas* maintained high peaks in zero and 6 days fruits (Figures 3a and b) compared with the very low peaks in 12 and 26 days fruits (Figures 3c and d). Generally, the population of all the organisms increased in storage. Lactococcus maintained high peaks in 6, 12 and 26 days (Figures 3b, c, d).

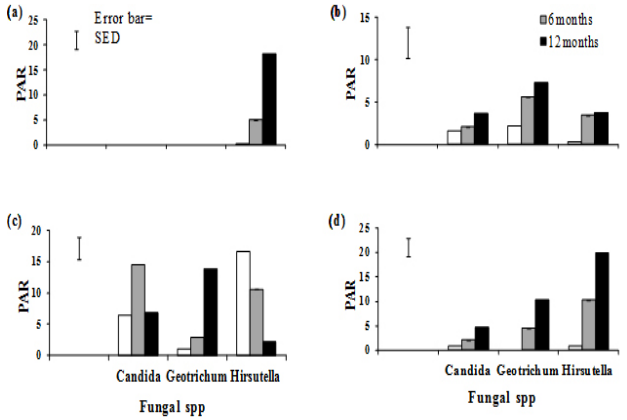

Figure 2. Peak area ratios (PAR) of persistent fungal spp. identified in palm oil processed from various fruits (a) 0 days, (b) 6 days, (c) 12 days, (d) 26 days. Oils were examined over a period of twelve months. Zero days represent the fresh fruits. SED = 0.423.

Table 1 represents the statistical analysis results of microbes identified in palm oil. The bacterial species can be grouped into four (a,b,c,d) based on their ratios (Table 1A). Overall, *Pseudomonas* had the largest population (3.52), followed by *Lactococcus* (2.69), with *Bacillus* having the smallest population (0.10), Table 1A. The length of storage of the oil samples were positively correlated (r = 0.929) with the bacterial load. Palm oil stored for 12 months had the largest bacterial load of 2.18, followed by those stored for 6 (1.59) and 0 months (1.17), Table 1A.

Table 1. Microbes identified in palm oil processed from various fruits and selected mills

Samples	Mean Bacteria peak ratio (load/pop)	Mean Fungi peak ratio (load/pop)	Bacteria spp	Bacteria spp peak ratio (load/pop)	Fungi spp	Fungi spp peak ratio (load/pop)	Storage period (months)	Total Bacteria peak ratio (load/pop)	Total Fungi peak ratio (load/pop)
A. Oil from stored fruits									
Fresh (0 days)	2.01 ***a***	1.17 ***a***	*Bacillus*	0.10 ***a***	***Candida***	1.94 ***a***	0	1.17 ***a***	2.13 ***a***
6 days	1.95 ***a***	2.70 ***b***	*Bordetella*	0.56 ***a***	***Geotrichum***	1.93 ***a***	6	1.59 ***b***	2.40 ***a***
12 days	1.11 ***b***	2.85 ***b***	*Lactococcus*	2.69 ***b***	***Hirsutella***	3.02 ***b***	12	2.18 ***c***	2.37 ***a***
26 days	1.53 ***c***	2.48 ***b***	*Leuconostoc*	1.19 ***c***					
			Pseudomonas	3.52 ***d***					
			Rhodobacter	1.73 ***c***					
			Waddlia	1.47 ***c***					
			Zymobacter	1.91 ***c***					
B. Oil from small-scale mills									
1	0.93 ***a***	0.43 ***a***	*Bacillus*	0.08 ***a***	***Candida***	1.34 ***a***	0	0.69 ***a***	-
2	0.86 ***a***	2.07 ***b***	*Bordetella*	0.09 ***a***	***Geotrichum***	1.00 ***a***	6	1.03 ***b***	-
3	1.37 ***b***	1.32 ***bc***	*Lactococcus*	1.83 ***b***	***Hirsutella***	2.11 ***b***	12	1.11 ***b***	-
4	0.58 ***ac***	8.04 ***d***	*Leuconostoc*	1.36 ***c***					
5	0.37 ***c***	1.01 ***ac***	***Pseudomonas***	1.30 ***c***					
6	0.66 ***c***	0.24 ***ac***	*Rhodobacter*	1.32 ***c***					
7	0.45 ***c***	0.41 ***ac***	*Ruminobacter*	0.23 ***a***					
8	0.53 ***ac***	0.56 ***ac***	*Waddlia*	1.33 ***c***					
9	0.68 ***ac***	1.02 ***ac***	*Zymobacter*	1.11 ***c***					
10	0.42 ***c***	0.80 ***ac***							
11	2.43 ***d***	2.32 ***b***							
12	1.09 ***ab***	1.31 ***bc***							

Fungi and bacteria spp identified in palm oil. For various fruits (A): Fprob value for bacteria spp * storage period = 0.001**; CV = 9.9%. Fprob value for fungi spp = 0.02*; CV = 30.1%. For oil (B): Fprob value for the oil samples and bacteria spp = 0.001**; CV = 27.7%. Peak area ratios with the same letter are not significantly different from each other. Those with different letters are significant. ** significant at 1%; * significant at 5%. Organisms in bold writing were identified by sequencing. Oil samples: (1) Adankrono 3; (2) Okumaning 3; (3) Okumaning 6; (4) Subi 3; (5) Nkwantanang 5; (6) Kusi 1; (7) Takrowase 1; (8) Kade 4; (9) Kade 11; (10) Wench 10; (11) Wench 12; (12) Large- scale oil mill (LSOM). Note high peak area ratio of fungi spp in sample 4.

Table 2. Changes in FFA (%) of stored palm oil processed from various fruits and selected mills

Samples	Storage period of fruits (Days)	FFA(%) of palm oil stored for months					Mean
		0	3	6	9	12	
A.	Oil from various fruits						
	0 (fresh)	0.45	1.33	2.74	2.74	2.99	2.05 ***a***
	6	6.02	5.19	6.17	5.90	6.26	5.50 ***b***
	12	11.34	12.42	13.44	13.31	13.95	12.89 ***c***
	26	32.37	34.43	36.09	35.46	36.48	34.97 ***d***
	Mean	12.04 ***a***	13.34 ***b***	14.61 ***c***	14.35 ***c***	14.92 ***cd***	
B.	Oil from small-scale mills						
1	9.0	5.95	-	8.07	-	9.60	7.87 ***a***
2	11.5	11.67	-	13.19	-	17.79	14.23 ***b***
3	16.0	18.46	-	19.59	-	25.47	21.17 ***c***
4	7.5	5.17	-	5.89	-	8.55	6.54 ***d***
5	7.0	4.64	-	5.57	-	6.25	5.49 ***e***
6	17.5	20.56	-	23.94	-	31,87	25.46 ***f***
7	12.0	11.87	-	14.21	-	25.86	17.31 ***g***
8	18.0	20.79	-	23.68	-	33.79	26.09 ***h***
9	7.0	4.91	-	5.76	-	7.81	6.16 ***d***
10	15.5	17.85	-	17.85	-	21.76	19.90 ***i***
11	15.0	16.13	-	19.84	-	20.74	18.90 ***j***
12	1.5	1.03	-	3.07	-	3.20	2.21 ***k***
Mean		11.53 ***a***	-	13.20 ***b***	-	17.72 ***c***	

Standard acceptable value for FFA ≤ 5% (PORAM Standard Specifications for Processed Palm Oil, 2011)

The means in Table 2 represent FFA values in oils stored over 12 months. Mean values with the same letters are not signi- ficantly different from each other. Those with different letters are significant. For fruits (A): Fprob for storage period*type of fruits < 0.006**, SED = 0.2595, CV = 0.2%. For oil (B): Fprob for storage period < 0.001**, SED = 0.5059, CV = 0.5%. ** significant at 1%. Oil samples: (1) Adankrono 3; (2) Okumaning 3; (3) Okumaning 6; (4) Subi 3; (5) Nkwantanang 5; (6) Kusi 1; (7) Takrowase 1; (8) Kade 4; (9) Kade 11; (10) Wench 10; (11) Wench 12; (12) Large- scale oil mill (LSOM). Note high peak area ratio of fungi spp in sample 4.

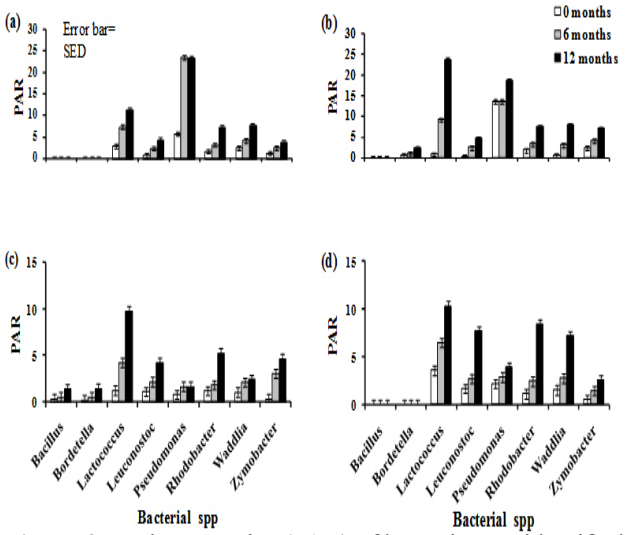

Figure 3. Peak area ratios (PAR) of bacteria spp. identified in palm oil processed from various fruits. Oils were examined over a period of twelve months. (a) 0 days, (b) 6 days, (c) 12 days, (d) 26 days. Zero days represent the fresh fruits.

Similarly, persistent fungal species in samples from the small-scale mills were *Candida*, *Geotrichum* and *Hirsutella*. *Hirsutella* was the most predominant species, with a peak area ratio and population of 2.11 followed by *Candida* (1.34) and *Geotrichum* species (1.00), Table 1B. Contrary to the results in Table 1A, dominant bacterial species in Table 1B is *Lactococcus* (1.83). The length of storage of oils from the small-scale mills was also positively correlated (r = 0.716) with the bacterial species. Palm oil stored for 12 months had the largest bacterial load of 1.11 compared with 0.69 for zero months (Table 1B).

Palm Oil Quality Analyses

Length of storage of fruits before processing into oil were positively correlated (r = 0.972) with the free fatty acid (FFA). FFA of oil processed from the fresh fruits did not change significantly even when stored for twelve months and still remained within the acceptable standard level of 5%. However, the deterioration (FFA) of all the oils from fruits stored for different periods were above the standard level of 5% (Table 2A). Apart from samples 4 (mean FFA of 6.54) and 9 (mean FFA of 6.16), FFA of all the samples from small-scale mills differ significantly from each other (Table 2B). It is observed that the longer the fruits and oil are stored, the higher the FFA and therefore the higher the deterioration.

The length of storage of fruits (0, 3, 6, 9, 12, 19, 26 days) before processing into oil did not have any influence on the moisture and impurity content of the oil because differences observed in the fruit types were not significant (results not shown) Moisture and impurity content of oils examined were above the standard levels of 0.01-0.04 and 0.1% for moisture and impurity respectively. Generally, oil from the large-scale oil mill (sample 12) had the lowest moisture content of 0.10 while sample 8 (from a small-scale mill) had the lowest impurity content (results not shown).

Fifteen fungi and fourteen bacterial species were identified in palm oil. Three fungal and eight bacterial species were persistent. The persistent fungi were *Candida, Geotrichum* and *Hirsutella* spp. while the bacterial species were *Bacillus, Bordetella, Lactococcus, Leuconostoc, Pseudomonas, Rhodobacter, Waddlia* and *Zymobacter* (Table 1). The high peaks of pseudomonas in oils processed from zero and six days fruits could possibly be due

to the succession of *Pseudomonas* arising from competition with other species in samples c and d (Fig 3). Comparatively more microbes were identified on palm fruits compared to the palm oil. This could be due to the fact that a substantial amount of these organisms may be eliminated from palm fruits when processing into the oil.

The high water activity in the palm fruits also supported more microorganisms than that in palm oil. Bacteria require a water activity (a_w) of at least 0.9 to grow whilst fungi require a minimum of 0.7 a_w. Water activity is positively correlated with the moisture content of the samples. Thus the higher the moisture content of the sample, the higher the water activity (Chirife *et al*., 2006). The total moisture content within the palm fruits over the 26 day study period was 23.75% which is equivalent to 0.76 water activity. Moisture content of the oil samples however ranged from 0.01 to 0.60% (Chirife *et al*., 2006; Tagoe, 2008). The moisture required for microbial growth is therefore far below the required percentage in palm oil.

Samples with large fungal populations have few bacterial species growing on them and those with small bacterial populations had a huge fungal load. Apart from *Lactococcus* and *Leuconostoc*, all the microbes identified in palm oil were also identified on the fruits. Micro-organisms identified in the oil include colonisers, lipolytic, soil and water inhabitants. *Zymobacter palmae* is one of the commonest bacterial species identified in the palm oil. It originates from the palm sap (locally called palm wine) and produces ethanol as a primary fermentation product from various hexose sugars and saccharides (Raj *et al*., 2002).

Eventhough FFA content of palm oil processed from the fresh fruits and the large scale mill remained within the standard level of 5%, it is obvious from the microbial analysis that the high-grade oils which have low FFA contain microbes. The longer the oil is stored, the higher the microbial load. Work done by Odunfa (1989) on the deterioration of Nigerian palm oil in storage showed that microbial attack leads to hydrolysis of the oil and hence the formation of free fatty acids. Idem (1973), Oso (1979), Airede and Esuruoso (1987) also showed that fungal infection of palm kernels increased the FFA content of the oil.

The free fatty acid (FFA) content of palm oil was determined by the storage period (days after harvesting) of the fruits before processing into oil and the storage period of the oil after processing. Fruits that have been stored for long periods prior to processing have high FFA contents. However, eventhough the rate of deterioration during storage is significant in both cases, changes during the twelve months are comparatively low when compared to changes related to the fruit storage. Palm oil processed from the fresh fruits had an initial FFA of 0.45% and this increased gradually as the length of storage increased. At twelve months the FFA was 3.00% which is encouraging because FFA still remained within the standard level of 5%. Oil processed from six-day old stored fruits however had an initial FFA of 6.02%, whilst 12-day old stored fruits had an initial FFA of 11.34% and 26 days had an initial FFA 0f 32.37%. These initial FFAs are well above the standard accepted level. Results obtained confirm reports that oil from over-ripe fruits contain high levels of FFA (Salunkhe *et al*., 1992).

The results in Table 2A are confirmed by those obtained from the mills (Table 2B). FFA of all the samples analysed increased with the length of storage time. Oil from the large scale mill (sample 12) had a mean FFA of 2.21% by the twelfth month. Samples 4, 5 & 9 from the small scale mills also had mean FFAs (6.54, 5.49 & 6.16%) close to the standard level. The others had initial FFAs well above the standard. For most samples from the small scale mills, storage period of the fruits used to process the oils could be estimated from the FFA levels to be at least 12-26 days old. These results confirm the findings by Wu and Bechtel (2008) on salmon by-product storage and oil extraction. The authors observed that the FFA composition and antioxidant activity in oil from aging pink salmon and vicera stored for four days increased with storage time and temperature.

The sample from the large scale mill (sample 12) had the lowest moisture content of 0.10% and Sample 6 from a small scale mill (results not shown) had the highest (0.75%) compared to the standard level of 0.01-0.04%. However, all the samples studied had moisture and impurity contents above the standard levels. This could possibly be due to the precision of the methods used. Eventhough significant differences occur between the samples and storage periods for moisture and impurity, these differences do not show any trend. For example, it is not clear whether moisture content is influenced by the length of storage of the oil or the type of fruits used to process the oil.

The moisture and impurity content of palm oil could be greatly influenced by the methods of processing (Poku, 2002). The large scale oil mills use electricity generated through turbines for heating purposes. Therefore, the processing operations are automatically controlled and routine sampling and analyses by laboratories ensure smooth and efficient operation. The small scale mills, however, use fire generated from fruit bunches or fuel wood for heating purposes during processing. The oil is boiled for 1-2

hours, allowed to settle, decanted, and reheated in cooking pots. The dried oil is then carefully skimmed off leaving any impurities at the bottom of the pot (Poku, 2002). Therefore, the more skillfully the process is carried out, the lower the moisture and impurity content of the oil.

Conclusion

The quality of palm oil is greatly influenced by the method of processing. The scale of operations differs at the level of processing and this affects the quality of the final product. The work shows that the free fatty acid content of palm oil is determined by two factors. The first and major factor is the length of storage of the fruits used to process the oil. The second factor is the length of storage of the oil after processing. Oils processed from the fresh fruits have a very low initial FFA (0.45%) content compared with those from 6 (6.02%), 12 (11.34%) and 26 (32.37%) day old fruits. Results also show that the high grade oils, i.e. those with very low FFA content, contain microbes and the longer the oil is stored, the higher the microbial load.

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