

THE EFFECT OF CRUDE LEAF EXTRACTS OF *MORINGA OLEIFERA* ON THE BACTERIAL, NUTRITIONAL AND SENSORY PROPERTIES OF WEST AFRICAN SOFT CHEESE

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The objective of this study was to assay the bacterial status and nutritional-cum sensory properties of West African Soft Cheese treated with 3 levels (1%, 2% & 3%) of ethanol and ether extracts of *Moringa oleifera* leaf, using a 2*4 factorial design. The results indicated that 2% and 3% moringa leaf ethanol extract had the highest inhibitory potential against bacteria population ($p < 0.05$). The proximate analysis revealed that 1% ethanol extract-treated samples had the highest levels of crude protein and ash while the control cheese had significantly higher moisture and fat content ($p < 0.05$). Sensory analysis of the various samples revealed that analysts preferred the cheese preserved with 3 % ethanol extract. It can thus be concluded that the use of ethanol extract of moringa leaf as a Cheese preservative leads to improved microbial stability and nutritional quality, as well as higher sensory acceptability.

Key words: cheese, *wara*, microbial, sensory analysis, *Moringa oleifera*.

Milk and dairy products, including Cheese continue to play an important role in the nutrition of people in many parts of the world. Cheese (hard Cheese) can be preserved for up to one year whereas other fermented milk products such as yoghurt can be preserved only for few days (FAO, 1990). West African Soft Cheese, commonly known as *wara* in Nigeria, however, has a relatively short shelf-life of 2 - 3 days when stored in whey at room temperature as is traditionally done (Adegoke *et al.*, 1992; Belewu *et al.*, 2005). Several attempts have been made to extend its shelf life. Adetunji *et al.* (2007) used lemon juice as a coagulant, Belewu *et al.* (2011) reported the

use of honey and Moringa oil to reduce the microbial contamination, while Belewu (2012) used the essential oils of lemongrass and eucalyptus to reduce the microbial load of Soft Cheese. Badmos and Abdulsalam (2012) compared the potency of thyme and honey as cheese preservatives. Biological preservation of West African Soft Cheese has been variously researched (Sanwal and Payasi, 2007; Belewu *et al.*, 2011; Adetunji, 2011). The properties of *Moringa oleifera* have similarly attracted the interest of researchers.

Moringa oleifera Lam., a highly valued member of family Moringaceae, is a fast growing plant widely available in tropics and subtropics with much economic importance for industrial and medicinal uses. *M. oleifera*, commonly called “miracle tree” is rich in nutrients and has been reported to have antimicrobial properties. It is described as one of the most useful plants in the world. It is cultivated in all countries of the tropics for its leaves, fruits, roots and for a variety of food and medicinal purposes (Fahey, 2005; Moyo, 2011). The leaves and other parts of this plant are now been dried, ground and sold as nutritional supplements in many countries. This is an option for harnessing the nutritional benefits that abound in this plant.

This work evaluates the preservative and antimicrobial potency of dried and powdered *M. oleifera* leaf, extracted with ethanol and petroleum ether.

MATERIALS AND METHODS

Moringa Leaf Extraction

Packaged Moringa leaf powder was purchased from the Moringa plantation unit of the University of Ilorin. 150g of Moringa

Table 1 : Chemical Composition of Dried Leaves of *M. oleifera* lam

Nutritive Value	Dry Leaf	Standard Error
Moisture (%)	9.533	0.194
Crude Protein (%)	30.29	1.48
Fat (%)	6.5	1.042
Ash (%)	7.64	0.433
Neutral Detergent fiber (%)	11.4	0.425
Acid Detergent fiber (%)	8.49	0.348
Acid Detergent lignin (%)	1.8	2.204
Acid Detergent cellulose (%)	4.01	0.101
Condensed Tannins (mg/g)	3.12	0.104
Total polyphenols (%)	2.02	0.39

Source : Moyo *et al* (2011)

leaf powder was soaked separately in 600 ml ethanol and in petroleum ether (Mashiar *et al.*, 2009), in air-tight containers for 3 days with occasional shaking (Mensah *et al.*, 2012). The mixtures were then filtered into clean beakers and allowed to evaporate in the oven at 60-70°C. The residues were stored in the refrigerator pending use (Akueshi *et al.*, 2002; Mashiar *et al.*, 2009).

Sodom Apple Juice Extraction

Medium sized Sodom apple stem were obtained from University of Ilorin road. Exactly 200 g of it was weighed, washed in clean water, crushed with a mortar; and sieved with a muslin cloth (Badmos and Joseph, 2012).

Preparation of Cheese

The cheese was prepared in the Biotechnology and Dairy Laboratory of the University of Ilorin, as described by Badmos and Joseph (2012) and put back into its whey. Three replicates were made of each treatment (Ashaye *et al.*, 2006)

Experimental Treatments

Treatment 1: 1% ethanol extracted Moringa Leaf

Treatment 2: 2% ethanol extracted Moringa Leaf

Treatment 3: 3% ethanol extracted Moringa Leaf

Treatment 4: 1% Ether extracted Moringa Leaf

Treatment 5: 2% Ether extracted Moringa Leaf

Treatment 6: 3% Ether extracted Moringa Leaf

Treatment 7: control – no Moringa extract

Total Bacterial Count

Samples were aseptically taken on days 0, 1 and 2, and the bacterial counts were determined (Fawole and Osho, 2007). Serial dilutions of individual samples in sterile distilled water were prepared by aseptically transferring 1g of cheese sample into 9ml of sterile distilled water, shaken thoroughly to disperse the Cheese, diluted four times and then plated on Nutrient agar plates. The plates were incubated at 37° C for 48 hours. The total number of viable bacteria cell colonies were counted for the three replications and the values expressed as colony forming units per gram (cfu/g).

Sensory Evaluation

The samples were examined by a panel of thirty (30) judges who are familiar with the sensory characteristics of Cheese, based on five characteristics, thus: colour, taste, aroma, texture and overall acceptability using a 9-point hedonic scale. The most acceptable Cheese was scored 9, and the most unacceptable scored 1 (Larmond, 1977; Badmos and Abdulsalam, 2012).

Proximate Analysis

The following tests were carried out three hours after Cheese production: moisture, crude protein, fat, and ash - according to the methods of AOAC (1990).

Statistical Analysis

The experiment was carried out based on a 2x4 factorial arrangement. Data obtained was subjected to Analysis of Variance, and the means separated with the Duncan Multiple Range Test (Steel and Torrie, 1980)

RESULTS AND DISCUSSION

Proximate Analysis.

For moisture content (MC) values, the most significant reduction in moisture was in the 1 and 2 % ether extract Cheese indicating that ethanol was effective in decreasing the moisture of the cheese. The effect of this is a longer keeping period as a result of decreased available moisture needed for the growth of spoilage microorganisms. These values are also significantly different from

the moisture values of the other cheese samples. For the crude protein, 2 % ethanol cheese was significantly lower than the other cheese samples and it appeared that higher concentrations of both extracts generally resulted in lower cheese protein value. These findings are in tandem with a previous study by Makkar and Becker (1996) which indicated that ethanol-extracted *M. oleifera* leaf had high content of protein. This may be as a result of the denaturation of the proteins by the polar solvents even though this trend was not very consistent. The high protein content of all treatments relative to the control could be due to the high protein in moringa leaves (Moyo, 2011). Similarly, the crude fat values were observed to decrease at higher levels of extract, as 1% level of both ether and ethanol extracts resulted in

Table 2: The Effects of the Moringa Leaf Extracts on the Nutritive Values of Various Cheese Samples.

TREATMENTS	1% Eth	2% Eth	3% Eth	1% Etr	2% Etr	3% Etr	Control	±SEM
Moisture Content (MC)	31.02 ^b	32.37 ^b	34.48 ^c	27.51 ^a	28.1 ^a	32.03 ^b	36.18 ^d	0.01*
Dry Matter (DM)	68.98 ^{bc}	67.63 ^{ab}	65.52 ^b	72.49 ^c	71.90 ^c	67.98 ^a	63.82 ^{ab}	0.98*
Crude Protein (CP)	20.70 ^c	18.50 ^a	21.15 ^{cd}	23.61 ^e	21.37 ^d	21.64 ^d	19.21 ^b	0.00*
Crude Fat (CF)	24.43 ^c	21.70 ^b	21.36 ^b	21.48 ^b	20.13 ^a	20.05 ^a	25.72 ^d	0.04*
Ash	4.34 ^b	4.51 ^b	4.15 ^b	5.39 ^c	4.83 ^{bc}	4.49 ^b	1.62 ^a	0.02*

*values within rows not sharing common superscripts are significantly different (P<0.05)

Trt – treatments; Etn/Etr –ethanol and ether extracts respectively

Table 3: The Effect of the Moringa Leaf Extracts on the Total Bacterial Count of the Various Cheese Samples.

TREATMENTS	Day 0 (10 cfu/g)	Day 1 (10 cfu/g)	Day 2 (10 cfu/g)	Average Count (10 cfu/g)
1% Ethanol Extract	8.05 ^f	1.89 ^b	1.85 ^b	3.93 ^{bcd}
2% Ethanol Extract	1.53 ^d	1.76 ^b	2.52 ^d	1.97 ^{ab}
3% Ethanol Extract	1.23 ^b	1.48 ^a	2.18 ^c	1.63 ^a
1% Ether Extract	0.45 ^a	5.00 ^{cd}	2.34 ^c	2.56 ^{abc}
2% Ether Extract	1.45 ^c	5.00 ^{cd}	3.15 ^a	2.12 ^{ab}
3% Ether Extract	6.75 ^e	5.20 ^d	2.73 ^e	4.84 ^d
Control	1.26 ^b	4.93 ^c	6.43 ^f	4.21 ^d
±SEM	0.00*	0.09*	0.07*	0.07*

*abc value within columns not sharing common superscripts are significantly different (P<0.05)

Cfu/g means Colony forming unit per gram

‡ Average of daily bacterial counts of triplicate readings over the duration of the experiments

Trt – treatments; Etn/Etr –ethanol and ether respectively

Cheese with high fat content and 3% ether extract produced Cheese with lowest fat content. It was observed that there was a significantly higher ash content in all treatments than the control. The high ash content could be due to the high ash content in moringa leaves. (Ramachandran *et al.*, 1980; Moyo, 2011). In addition, it may also be partly because the solvents had already removed fat and water from the Cheese, thus increasing the density of the Cheese, and the amount of minerals per gram of Cheese analyzed. However, while there was no particular trend observed in the ethanol treatments, the ash content was lowest with 3 % ethanol extract ($p < 0.05$).

Total Bacterial Count:

The control Cheese increased in bacterial count throughout the three days of storage. The Cheese from the levels of ether extracted Moringa leaf increased in TBC after the first 24 hours, after which it drastically reduced. The 3 % ethanol extract of *M. oleifera* appears most inhibitory of bacterial growth. Cheese from ethanol extract levels had the least TBC throughout the days of storage, although the bacterial counts increased gradually for the days of storage.

This result is in accordance with the study Bukar *et al.* (2010) reported that ethanol extract of Moringa Leaf had the broadest spectrum of activity on the test bacteria while Devendra *et al.* (2011) reported that moringa leaves extracted with petroleum ether did not show any zone of inhibition against all tested bacterial strains. In the study by Usman *et al.* (2012), it was observed that ethanol extract of *M. oleifera* leaves have significantly better anti-inflammatory properties compared to the other extracts (ether, chloroform and distilled water) used in the research. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction (Cowan, 1999). Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in studies like this that require consumption. Ethanol is a good

solvent for polyphenol extraction and is safe for human consumption (Shi *et al.*, 2005).

It was observed that 3 % ethanol extract showed most significant inhibition of the bacterial population compared with the other treatments and the control, resulting in the average lowest bacterial count of 1.63×10^6 cfu/g of cheese. The least effective treatment was 1% ether followed by 1% ethanol. The lowest extract levels thus had the least anti-bacterial effect, which was in line with expectations. There have been studies that link proteins with antimicrobial properties. Dahot (1998) reported that *M. oleifera* leaf extracts contain small peptides which could play an important role in the plant's antimicrobial defense system. The proteins/ peptides are believed to be involved in a defense mechanism against phytopathogenic fungi by inhibiting the growth of micro-organisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Chuang *et al.*, 2007). Devendra *et al.* (2011) reported that moringa leaves extracted with petroleum ether didn't show any zone of inhibition against all tested bacterial strains. Bukar *et al.* (2010) also showed that moringa leaf ethanol extracts had the broadest spectrum of activity on the test bacteria. These reports corroborates the results of this study. Overall, the Cheeses with ethanol treatments had lower bacterial counts than those with ether treatments and this implies that for the purposes of this study, ethanol was a better preservative than Petroleum ether. It appears that the ethanolic preservation of the cheese has improved the nutritional profile of the Cheese in addition to preventing quick spoilage by bacteria.

Isolation and Identification

As shown in Table 4, the Control Cheese had all the bacteria except *Lactobacillus casei* and *Corynebacterium spp.*, 1% ether had the highest inhibitory property among the *M. oleifera* ether extract, while 2% ether extract had the least inhibition of bacterial growth. The 3% ethanol extract inhibited the growth of all the bacteria except *Lactobacillus casei*, *Lactobacillus spp.* and *Corynebacterium spp.* which were present in all other extract level. *Staphylococcus*

Table 4: Summary of the Effect of the Moringa Leaf Extracts on the Bacteria Colonies Isolated.

Isolates	1%Et n	2%Et n	3%Et n	1%Etr	2%Etr	3%Etr	Contro l
<i>Enterococcus</i>	-	+	-	+	-	+	+
<i>Corynebacterium kutscheri</i>	+	+	+	+	+	+	+
<i>Lactobacillus casei</i>	-	+	+	+	+	-	-
<i>Corynebacterium spp</i>	+	-	-	+	+	+	-
<i>Bacillus species</i>	-	-	-	-	+	+	+
<i>Bacillus pasteurii</i>	-	-	-	-	+	-	+
<i>Corynebacterium xerosis</i>	-	-	-	+	+	-	+
<i>Staphylococcus spp</i>	+	+	-	+	+	+	+
<i>Staphylococcus aureus</i>	-	+	-	+	+	+	+
<i>Lactobacillus sp</i>	-	+	+	-	-	-	+

species were present in all the Cheeses preserved with ether extract but absent in the (1 and 3 %) ethanol extract Cheeses. The 3% ethanol extract inhibited *Staphylococcus* species. Ethanol extract showed higher inhibitory potential against *Staphylococcus* species than ether extract, which is in line with a study by Karthy (2009) that antibacterial activity of ethanol showed considerable efficacy when compared with petroleum ether extracts against all the MRSA (Methicillin resistant *Staphylococcus aureus*) isolates used in the study. Ethanol extracts showed more inhibitory potentials against bacteria than the petroleum ether extracts. *Lactobacillus casei* and some other *Lactobacillus spp.* have been reported to show antagonistic property which produces bacteriocins which are effective against certain strains of bacteria (Saranya and Hemashenpagam, 2011). *Lactobacillus spp.* were observed in Cheese of all levels of the ethanol extract but absent in all the levels of the petroleum ether extracts except for *Lactobacillus casei* which was observed in 1% and 2% ether extract. This may have contributed to the inhibition of the growth of other bacteria in the ethanol extract (Saranya and Hemashenpagam, 2011).

Sensory Analysis.

From the results of the sensory analysis (Figure 1), the control cheese was poorest in colour rating ($p < 0.05$). This was perhaps because the pigments in the extracts

complemented the colour of the treated Cheeses. The same trend was observed for the taste evaluation.

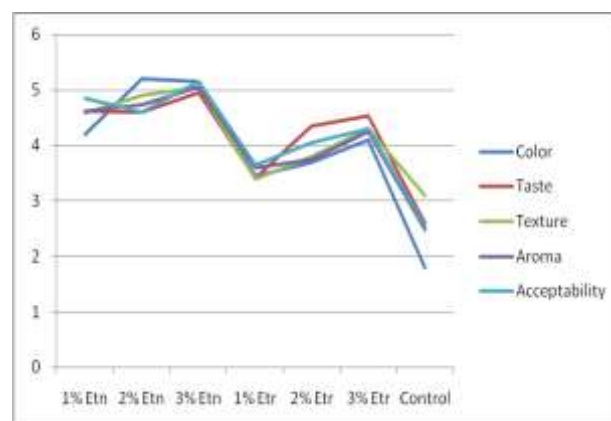


Figure 1: Effect of Moringa Leaf Extracts on the Organoleptic Properties of Cheese Samples.

The taste of the control Cheese was poorer than the treated Cheeses. Cheese treated with 1 % ether also had poor taste rating. This may be due to the limited amount of minerals (salts) extracted from *M. oleifera* leaf. The Cheese treated with 3 % ethanol extract gave the highest Cheese taste rating. The aroma and texture ratings of ether extract Cheeses were also found to be poor compared to the ethanol treatments, although more acceptable than the control. The Cheese texture, aroma and acceptability all followed the same trend with the colour and taste. Ethanol is found in limited quantities

in soft drinks which are widely consumed (Logan and Distefano, 1998), and the taste is familiar. This familiarity may be the reason for the higher acceptability of the ethanolic treatments compared to Cheese treated with petroleum ether extract as well as the control.

CONCLUSION

Cheese samples preserved with ethanol extracts of moringa leaves had the lowest microbial count when compared with those of ether extracts and control. They also had the highest crude protein and ash content, but lowest crude fat and moisture content, while they were also the most acceptable to consumers. It is thus recommended that (3%) ethanol extract of *M. oleifera* may be used in the preservation of West African soft cheese.

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