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EVALUATION OF DIFFUSSIVITY AND ACCEPTABLE MIXING RATIO FOR PRODUCTION OF HIBISCUS-MORINGA TEA BAGS

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Abstract
This study was conducted to evaluate the diffusivity and mixing ratio of hibiscus/moringa in tea bags.Hibiscus and moringa leaves were mixed at different ratios and labeled as samples A (70:30), B (60:40), C (50:50), D (40:60) and E (20:80). Lipton tea was used as control sample. Standard methods were used to determine the proximate compositions, pH, total soluble solids, and diffusion rate. Twenty (20) semi trained panelists conducted the sensory evaluation tests. The results of the proximate analysis show that the protein content ranged from 20.13 to 32.73 g/100g; increasing with increase in moringa content, the moisture content ranged from 3.05to 3.97 g/100g, the crude fibre content ranged from 10.13 to 11.93 g/100g. The ash content ranged from 9.70 to 11.14 g/100g, the fat content ranged from 9.67 to 10.24 g/100g. The result of the brix content ranged from 1.80 to 3.00 ^{o}Brix. The pH of the leave blends ranged from 3.30 to 4.06 with sample D having the highest pH and sample B having the lowest.The result of the diffusion rate ranged from 0.39 to 0.60 g/min; sample with 60% moringa and 40% hibiscus composition having the highest. The sensory evaluation shows that sample E (70% moringa and 30% hibiscus) was most preferred in terms of appearance, taste and general acceptability. The results of the study show that sample C (50% moringa and 50% hibiscus) had no significant difference in colour and general acceptability to control and sample E which was most accepted among all the samples, sample C has a significant different value of protein to sample E Sample C was therefore recommended for consumption for the amelioration of the incidence of protein malnutrition in developing countries.

Key words: Herb, tea, hibiscus, moringa, Brix, blend, diffusivity

1. Introduction

Tea is the processed leaves which upon infusion with cold or hot water, gives a non-alcoholic beverage (Sharma *et al.*, 2005). Tea consumption is an ancient habit, that originated from China and India dated back to about four thousand years (Chambers and Lee, 2007). Tea is currently the most widely consumed beverage in the world (Schmidt *et al*., 2005) and therefore ranks as an important world food product. It is generally consumed for its attractive aroma and taste as well as the unique place it holds in the culture of many societies. Traditionally, tea is consumed to improve blood flow, improve resistance to diseases and eliminate toxins (Choi *et al*., 2000). In recent times, there is renewed interest in tea because of growing consumer awareness of health benefits derived from its consumption (McKay and Blumberg, 2002). Tea therefore belongs to a rapidly expanding market of 'wellness beverages' (Byun and Han, 2004). Several authors reported that tea consumption can be linked to reduction of the risk of cardio vascular diseases, high cholesterol levels, diabetes, arthritis, osteoporosis and dental carries (Choi *et al.* 2000; Chamber and Lee, 2007). Teas have been traditionally categorized into green and black teas according to the processing conditions employed during manufacturing (Kirk and Sawyer, 1997). In recent times, however, a fourth category, called herbal teas, is gaining increasing popularity among consumers. Unlike traditional teas, herbal teas are prepared from plants other than Camellia (Bender, 2003). Herbal tea, according to many, looks like tea and is brewed as the same way as tea, but in reality it is not considered a tea at all. This is due to the fact that they do not originate from the *Camellia sinensis*, the plant from which all teas are made (Kumar *et al.*, 2005).Herbal teas are actually mixtures of several ingredients, and are more accurately known as 'tisanes.' Tisanes are made from combinations of driedleaves, seeds, grasses, nuts, barks, fruits, flowers, or otherbotanical elements that give them their taste and providethe benefits of herbal teas**.**Unlike most other forms of tea, herbal teas do not containcaffeine. They also taste great and are easy to drink. Most herbal teas may consist of one main herbal ingredient or a blend of herbal ingredients, intended to bring about aspecific purpose, such as relaxation, rejuvenation, relieffrom a specific condition, amongst other things **(**Aoshima *et al.,* 2007). Tea preparation follows a simple procedure. Hot water (70 to 100 °C) is poured over the plant part(s) in a container and allowed to steep for a few minutes (usually 1 –5 min) after which the plant material, usually contained in a bag, is removed from the container. The temperature of the water used and the duration of steeping affect the 'strength' of the tea. Tea is drunk hot, warm or iced. In some cases, milk and/or a sweetener such as honey or sucrose may be added before drinking (Hakim *et al.,* 2000). According to Abbey and Timpo (1990), indigenous herbs are in general heavily under-exploited in spite of their huge dietary potential. It is therefore imperative to explore the potential of indigenous plant materials in the development of new herb tea.

Moringa leaf is rich in minerals, amino acids, vitamins and carotene. It also contains a rare combination of health-promoting antioxidants: zeatin, quercetin, sitosterol, caffeoylquinic acid (Anwar *et al.*, 2007). Currently, there is growing interest in the use of Moringa leaf as an ingredient in the preparation of herbal tea. It may be necessary to combine Moringa with other herbs in developing herbal tea as a way of improving its sensory appeal. This is crucial because consumers are generally unwilling to buy food with poor sensory appeal, irrespective of health or nutritional benefits (de Cock *et al.,* 2005).

Roselle *(Hibiscus sabdariffa)*is an aromatic, astringent herb with multiple food uses including th e preparation of beverages. Roselle is known to impart a characteristic reddish colour and sour ta ste which many consider appealing in beverages (Blench, 1997). Roselle is an annual herb that is grown in the tropics and it is widely cultivated in Nigeria mainly in the North-Eastern and Middl e-belt regions. It contains anthocyanins, sugar and is used for medicinal purposes but it is widely used in making a fruit drink popularly known as 'Zobo' in Nigeria. The roselle calyx is susceptib le to decomposition thus it is essential to dry it. Drying is probably the oldest and the most impor tant method of food preservation practiced by humans, it is one of the main post-harvest operatio ns for biological materials since it has great effects on the quality of the dried products by mainta ining the nutritional properties basically the ascorbic acid content, it reduces the moisture content from 86% to 16% or 14% for improved preservation which is currently done by direct exposure of the calyx to the sun. The objective of this study is to determine a mixing ratio of Moringa and hibiscus leaves that will give a very acceptable drink from the leaves.

2. Materials and Methods

2.1 Source of materials

Fresh Moringa leaves were harvested from Olukoya Farms in Ile-Ife, Osun State. Moringa was harvested at about ten (10) cm from the tip of the leaves and this included leaves and petioles of the plant. All wilting and visibly diseased plant materials were manually removed. Hibiscus leaves were bought from Oba market, Akure, Ondo State.

2.2 Samples Preparation

All plant materials were carefully inspected and all foreign materials removed. Diseased and damaged leaves were discarded manually just after the collection of fresh leaves. Moringa and Roselle were not cut into pieces, and the leaf stalks of Moringa were not removed.

Moringa and Hibiscus leaves were washed in running tap water to remove dirt. After this, leaves were soaked in 1% saline solution (NaCl) for 5 minutes to remove microbes. The leaves were further washed with 70% ethanol followed by twice washing with distilled water. This step played a substantial role in removal of dust, pathogens as well as microbes present on the leave surface.The leaves were air dried by spreading on sterile clean green net in a well ventilated room to prevent loss of nutrients through exposure to high temperature for 4 days. After drying, the leave samples were milled using an attrition mill. The machine was washed before and after milling of each sample. The milled material was sieved through an Aluminum sieve (2mm aperture). The sieved samples were stored in glass bottles with tight lids and labeled.

2.3 Drying of the leaf powders

Moringa and Hibiscus leaf powders were further dried at 50 ^oC in a cabinet dryer (model 85mo64 Shandom, UK) for 30 minutes to reduce moisture content to 10% to enhance increased shelf life. The leaf powders were stored separately in clean air-tight containers, protected from light and humidity, and kept in a refrigerator to maintain a temperature below 24°C (75.2 °F).

2.4 Mixing of the blended samples

Each of the leaves sample was weighed into a Kenwood blender for proper mixing in the ratios as shown in Table 1 to get five samples. They were bagged in non-drip tea bags after operating the mixer for three minutes for each blend using a stay material and sealing machine. Each tea bag contained approximately 3 g of the blended samples. The tea bags were stored in glass bottles with tight lids and labeled for analyses.

Table 1: Proportion of herbs in blended products

Sample code	Moringa leaves (%)	Hibiscus leaves (%)
A	70	30
B	60	40
C	50	50
D	40	60
E	20	80

2.5 Determination of Proximate Composition

The proximate analysis (moisture, fat, ash, protein, carbohydrate and fibre) of the fresh leaves and products was carried out using AOAC (2010) method. However, carbohydrate content was determined by the difference method. The tests were carried out in triplicates.

2.6: pH

The pH values of the samples were measured weekly and directly using a pH meter (pHS 25). Five grams (5 g) of each sample was first dissolved in 50 cm^3 distilled water in a beaker and thoroughly shaken (Ibitoye, 2005). The pH meter was standardized using buffer solutions pH 4 and 7. The values were taken.

2.7: Brix

The glass slide of the refractometer (Atago hand refractometer N1 0-32%) was cleaned with water and wiped dry with a clean napkin. A smear of the sample was made on the slide of the refractometer and the lid replaced. The reading was taken at the graduated mark. This reading indicates the total soluble solids value of the sample and was recorded in degree brix (^{o} brix) (Owoso*et al*. 2000).

2.8: Diffusion rate

One tea bag of each sample was soaked for 5 minutes in 25 cl of water pre-heated to 100 ^{o}C. Each tea bag was being removed and dried in the oven until no further drying took place, each was then weighed. The diffusion rate was calculated using equation 1.

$$\frac{w_0 - w_1}{5} = DR \qquad (1)$$

where: w_o = initial weight of each tea bag (g), w_1 = final weight of each tea bag (g), D_R = Diffusion rate (g/min)

2.9: Sensory Evaluation

Sensory evaluation was conducted 2 (two) days after processing by 20 semi trained panelists (12 females and 8 males, between the ages of 20 to 26). The established sensory profile included 10 descriptors with their definitions and evaluation techniques (Tsai *et al*., 2002). All the panelists were briefed before evaluation. Sensory attributes like appearance, colour, taste, aroma, after taste and overall acceptability for all samples were assessed using nine point hedonic scales. Hedonic scale was in the following sequence: 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2= Dislike very much and 1= Dislike extremely. The samples were coded with letters and served to the panelists at random to guard against any bias (Chen *et al*., 1998) and samples were evaluated on three separate occasions. Drinking water was provided for palate cleansing after each evaluation.

2.10: Statistical Analysis

All determinations were carried out in triplicates. Descriptive statistics, analysis of variance (ANOVA) and Duncan Multiple Range Test were used to interprete the results obtained, and the level of significance was set at $p \leq 0.05$.

3.0: Results and Discussion

The result of the proximate composition of the leave blends is presented in Table 2. Sample A had the highest protein content (32.73±0.18 g/100g) indicating that the moringa leaf is a good source of protein. From the results, an increase in the proportion of moringa leaf significantly increased the protein content of the final product. This is an indication that consumption of Moringa leaf can help in eliminating the prevalence of Protein Energy Malnutrition (PEM) amongst consuming groups (Appel, 2003). The moisture content ranges from 3.05±0.05 g/100g to 3.97±0.15 g/100g.The observed low moisture content in the processed leaves could be due to the effect of processing techniques (Olitino *et al.*, 2007). The lower moisture observed in this study is an indication that the activity of the microorganisms would be reduced and thereby increased the shelf life of the flour samples. This observation is in agreement with the report of Olitino*et al.*, (2007). The result for the moisture content is also a useful indication of the possibility of the production of products with longer shelf life and extensibility (Caceres *et al.*, 1991). Samples A, C, D and E are not significantly different at (p< 0.05). The crude fibre content also ranged from 10.13±0.13 to 11.93±0.02g/100g. The fibre content of sample B and D are not significantly different from each other. The ash content of the blend of moringa and hibiscus leaf flours ranges from 9.70 to 11.14g/100g. Sample D had the highest ash content (11.14 g/100g) followed by sample B (10.91 g/100g) indicating that both samples are good sources of essential minerals. The ash content of about 11% indicates that the leaves are rich in mineral elements. Ash content is a reflection of the mineral status, even though contamination can indicate a high concentration in the sample (Baah *et al.*, 2009). The fat content in the blend of flour ranges from 9.67 to 10.2g/100g. Samples A and C had the lowest fat content (9.67 g/100g) due to the presence in smaller proportions of the hibiscus powder. From the results obtained, it can be said that the hibiscus flour contributed more to the fat content of the blend than the moringa flour. The carbohydrate content ranged from 32.39 to 46.17 g/100g. Sample E had the highest carbohydrate content (46.17 g/100g) as a result of high proportion of the hibiscus powder in the sample. From the results, it can be deduced that the hibiscus flour is a good source of carbohydrate. The chemical composition values confirmed that M. oleifera and hibiscus leaves powders are excellent food sources justifying its direct use in human nutrition or development of balanced diets for animal nutrition and use of this sample for tea bag instant drink for enhancement of nutrient composition in the diet.

Table 2: Proximate composition of the sample blends

PARAMETER	**Samples**				
	A (g/100g)	B (g/100g)	C (g/100g)	D (g/100g)	E (g/100g)
Crude protein	32.73 ±0.18[e]	31.24±0.09[de]	26.08±0.01[c]	23.28± 0.18[b]	20.13±0.18[a]
Moisture	3.43±0.04[b]	3.31±0.01[bc]	3.05±0.05[bc]	3.97±0.15[c]	3.31±0.00[b]
Ash	9.70±0.16[a]	10.91±0.07[ab]	10.76±0.01[bc]	11.14±0.01[a]	10.36±0.41[ab]
Fat	9.67±0.02[a]	10.24±0.26[b]	9.67±0.02[a]	10. 02 ± 0.03[ab]	9.83±0.02[ab]
Fibre	10.54±0.35[b]	11.93±0.02[d]	11.65±0.08[c]	12.03±0.03[d]	10.13±0.13[a]
Carbohydrate	33.95±0.07[b]	32.39±0.43[a]	38.85±0.07[c]	39.58±0.08[c]	46.17±0.70[d]

Means with the same superscript in the same column are not significantly different (P>0.05)

The result for the sensory evaluation of the Moringa and Hibiscus sample blends are presented in the Table 3 below. Form the results, it can be observed that sample E had the most preferred appearance followed by sample C according to consumer evaluation of the samples. The appearance of the control sample is also preferable when compared with sample B, in terms of appearance. Sample A had the least appearance preference which indicated that the product was not widely acceptable by consumers. A reason for the appearance of sample A might be as a result of an increase in the ratio of the Moringa powder compared to the Hibiscus powder (Fasoyiro *et al.*, 2005). Sample E had the highest consumer acceptability as regards the colour of the sample. According to (Tsai and Huang, 2004), the colouring component of the Hibiscus flour have been observed to be anthocyannins and the stability and degradation of the brilliant red colour of the leaf is dependent on the pH. It has been observed that the colour intensity in Hibiscus flour is also favoured by the low pH as anthocyannins have little colour above pH 3.5 (Bridle and Timberlake, 1997). Unlike sample A which panelists showed a little dislike in the colour acceptability, samples B, C and D are preferred.. The colour of the control sample is also slightly acceptable but shows little difference from the samples B, C and D. Sample A is the least preferred sample in terms of colour acceptance by consumers. This can be attributed to the increase in the ratio of the Moringa to the hibiscus powder.

Sample E was better accepted in term of taste when compared to all other samples and this can be as a result of the increase in the ratio of the Hibiscus. Consumers showed a very high degree of dislike in the samples A and D in terms of taste and this can be attributed to the high amounts of moringa in the samples. The control sample also tasted better than any of sample B or C and can be compared to sample E. From the result obtained, it shows that consumers will prefer both sample E and control because of the high amounts of Hibiscus flour which is naturally sweet. The results from the after taste of the samples show that sample E had the most preferred after taste compared to the other samples. This is because of the presence in higher proportion of the Hibiscus powder. As a result, consumers will prefer sample E better than other samples. It can be said that the increase in the ratio of the Hibiscus is responsible for the sweetness of the final products (Fasoyiro *et al.*,2005). Samples which have slightly lower amounts of Hibiscus powder and higher amounts of Moringa powder were not preferred by the panelists. The control sample (Lipton) had the highest acceptable aroma amongst all other samples. This is followed by sample E as a result of the higher proportion of hibiscus powder. Samples with lesser values of hibiscus have little consumer acceptability. Samples A, B, C, and D are not significantly different from each other. This can be attributed to the higher proportion of the moringa powder (Shin, 1994).

Results from the sensory evaluation revealed that the samples B, C, and E are generally acceptable. The samples were preferred to others when compared because the hibiscus powder had a positive influence on the sensory attributes. Consumers will prefer the final products which have higher proportion of hibiscus powder in the ranges between 50, 60 and 80.

Table 3: Sensory evaluation result

SAMPLE	COLOUR	AROMA	TASTE	AFTER-TASTE	APPEARANCE	ACCEPTANCE
A	5.50 ± 1.58^{c}	5.40 ± 1.07^{b}	4.70 ± 1.25^{c}	3.30 ± 1.34^{c}	5.40 ± 0.97^{b}	5.20 ± 1.48^{b}
B	6.70 ± 0.82^{b}	4.80 ± 1.40^{b}	4.60 ± 1.84^{c}	5.40 ± 1.71^{ab}	5.30 ± 0.67^{b}	5.90 ± 0.57^{b}
C	6.90 ± 1.10^{b}	5.80 ± 1.14^{b}	5.60 ± 1.71^{bc}	6.20 ± 1.40^{a}	6.90 ± 2.08^{a}	7.50 ± 1.43^{a}
D	7.60 ± 0.97^{ab}	5.80 ± 0.42^{b}	5.50 ± 2.17^{bc}	4.30 ± 1.64^{bc}	6.00 ± 0.82^{ab}	7.30 ± 0.95^{a}
E	8.30 ± 0.95^{a}	7.40 ± 0.84^{a}	7.40 ± 1.17^{a}	6.50 ± 1.43^{a}	6.90 ± 0.88^{a}	7.70 ± 0.95^{a}
CONTROL	6.60 ± 1.43^{b}	7.50 ± 1.43^{a}	6.70 ± 1.83^{ab}	3.80 ± 1.40^{c}	6.80 ± 1.48^{a}	7.90 ± 0.88^{a}

Means with the same superscript in the same column are not significantly different (P>0.05)

The result for the physico-chemical analysis of the leaves blend is presented in Table 4. The brix content of the leaves blend ranged from 1.80±0.10 to 3.00±0.20. The results obtained for the brix vary significantly except for samples A and E which are not significantly different from each other. The result obtained for sample D shows that the brix content is dependent on the proportion of moringa powder in the blend and that a high proportion of sugar molecules are observed in the sample. The results obtained for the brix of the leaves blends are higher than that reported by Rita de Cassia *et al.* (2014) which presented 1.5°Brix for moringa seeds, 1.9°Brix for the leaves and 0.2°Brix for polymer.

The pH values of the leaves blend were higher for the more concentrated samples. It was observed from the results that the sample with the highest brix content had the highest pH value.

Samples that are more concentrated with moringa powder tend to have elevated levels of acidity. The results obtained can be compared to the pH 3.13-3.28 reported by Bolade *et al.*, (2009). The result for pH reported by Rita de Cassia *et al.* (2014) also gave a pH value of 5.80 for the moringa seed, 5.15 for the leaves and 6.50 for polymer. The results obtained are higher when compared to all other samples indicating that the samples are slightly acidic in nature. This can be as a result of the presence of the hibiscus powder which increased the acidity of the moringapowder deviating from pH 6.3 to 7.0 for moringa powder. The acidity of the samples increased with increasing ratio of hibiscus powder.
Solutions with a pH less than 7 are acidic and solutions with a pH greater than 7 are basic. Pure water is neutral, being neither an acid nor a base. The pH of the leave blends ranged from 3.30±1.10 to 4.06±0.05 with sample D having the highest pH and sample B having the lowest value. The pH value of a substance refers to the acidity and alkalinity of the substance.

Table 4: Brix and pH result

Sample Blends	Brix	Ph
A	1.96 ± 0.25^{bc}	3.76 ± 0.15^{b}
B	3.00 ± 0.20^{a}	4.06 ± 0.05^{a}
C	2.16 ± 0.15^{b}	3.90 ± 0.10^{ab}
D	1.80 ± 0.10^{c}	3.57 ± 0.05^{c}
E	2.30 ± 0.15^{bc}	3.30 ± 1.10^{d}

Means with the same superscript in the same column are not significantly different (P>0.05)

Diffusion is the movement of a substance from an area of higher concentration to an area of lower concentration. There are lots of tea molecules in the bag and none outside. The leaves themselves can't pass through the bag but their smaller particles containing colour and flavour can (the teabag itself acts as the partially permeable membrane). The addition of heat (from the hot water) to the tea bag causes its molecules to move much faster than at room temperature. This energy is more readily released in a shorter period of time than a tea bag filled with room temperature or cold water. In this study, the diffusion rate of the samples ranged between 0.36 and 0.60 g/min which shows that sample B has the highest diffusion rate. But the result as shown in Table 5 has no direct correlation with the blends ratio.

Table 5: Diffusion rate result

SAMPLE	DIFFUSION RATE (g/min)
A	0.39
B	0.60
C	0.43
D	0.36
E	0.46

3. Conclusion

This study focused on the evaluation of the diffusivity and acceptability of zobo/moringa tea in bags and from the results obtained, it can be concluded that sample A (70:30) moringa/ hibiscus has the highest protein content but sample C (50:50) moringa/hibiscus was more acceptable than A in terms of general acceptability and appearance which is not significantly different from samples D, E and the Control. Since the protein content of sample C is relatively high when compared with samples D and E, Sample C is therefore recommended based on the results from this study. It is sufficient in improving protein malnourished diets thereby contributing to the reduction of protein energy malnutrition.

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