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Research Paper

Sensory Acceptability And Microbia Quality Of Chocolate Produced From New Nigerian Cocoa Hybrid.

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Abstract
A study was conducted on the sensory acceptability and microbial quality of chocolate produced from different cocoa hybrids (TC_1, TC_2, TC_5, TC_6 and TC_7) from Cocoa Research Institute of Nigeria (CRIN). The cocoa were processed into chocolate from the bean's liquor of the different cocoa hybrid using standard methods. The sensory acceptability and microbial quality of the chocolate were determined using standard methods. All the result was subjected to statistical analysis and the mean were separated using statistical package (SPSS 21.00). The sensory acceptability result revealed that chocolaty flavor, colour, texture, taste, mouth feel and overall acceptability values ranged from 5.80 to7.43, 5.73 to 7.35, 5.93 to 7.25, 4.95 to 6.50, 6.02 to 7.28 and 6.20 to 7.67 respectively. The result of the microbial quality shows that bacteria viable count, total viable count of staphylococcus aureus and the viable count of Escherichia coli ranges from 11.0 to 24.00 × 10^6, 2.0 to 6.0 × 10^6 and 3.0 to 4.0 × 10^6 cfu/g. The morphological characteristic of bacteria isolates from chocolate samples reveals colour of the colonies to be white, grey-white with size range from 1-5mm, majority were round in shape, flat, slightly raised with smooth edges, opaque with wet surfaces while fungi isolates were cotton-like, black and green filamentous. There was significant different ($p< 0.05$) among the samples of chocolate in all the sensory attributes evaluated and all were acceptable on a 9-point hedonic scale. The absence of certain pathogenic organism in samples TC1 and TC7 is an evident of stability and safety of the chocolate.

Keywords: *Microbial quality, Cocoa hybrids, Sensory acceptability, Chocolate, Bacteria, Staphylococcus aureaus, Echerichia coli,*

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I. IINTRODUCTION

Theobroma cacao was originally from Latin America and was transplanted to West Africa in 1824 by the Portuguese. The pod of cocoa contain 30 to 40 bitter seeds embedded in a sweet sticky pulp which has been a staple food for the Mayans 2000 years ago and the seed as chocolate drink [1], [30]. Sensory evaluation is a scientific discipline that analyses and measures human responses to the composition of food or product made by the senses of taste, smell, touch and hearing when food is eaten [2]. Cocoa has a microbial succession of a wide range of yeast, lactic acid and acetic acid bacteria during which microbial products such as ethanol, lactic acid and acetic acid are produced [1]. It is the major ingredient of cocoa based candies like chocolate which is presently being consumed for health reasons because of the presence of flavonoids in it [3]. Although the physiological roles of the predominant microorganisms were well understood and the crucial importance of a well-ordered microbial succession in cocoa aroma has been established [1]. Food safety is usually determined by the presence or absence of pathogenic organisms, or their toxins and the number of pathogenic with their expected or destructive agents. The level of spoilage microbes reflects the quality, wholesomeness of a food product as well as the effectiveness of hygiene.

II. METHODOLOGY

2.1 Production of Chocolate from Fermented Cocoa Beans

The chocolate was produce from the fermented cocoa varieties using the method described by [5], [6]. The chocolate samples (high cocoa content) were obtained using the following proportions cocoa liquor (63.0%), cocoa butter (7.0%), sugar (29.60%) and lecithin (0.4%) and were prepared in Cocoa institute of Nigeria, CRIN Ibadan, Nigeria. The seeds were triturated to remove the peel and germ in order to obtain cocoa nibs [31]. The nibs were ground m a knife-grinder, with sugar added m this phase The cocoa paste was then refined in a grounderro11er, yielding a cocoa paste with an adequate granulation (21 µm) for making chocolate The refined paste was subjected to conching in a horizontal shell at 60 °C.

2.2 Sensory Analysis of the Chocolate Sample

Qualitative (Preference) test was used on 30 panelists to indicate their preference on a 9-point hedonic scale where 1 and 9 represent dislike extremely and like extremely according to [7].

2.3 Determination of Microbiological Quality of Chocolates

The microbiological stability of the chocolate was determined by method described by [8] on a weekly basis for 3weeks. Determination were total aerobic plate count, *Escherichia coli* count, *Staphylococcal* count, *Salmonella* and *shigella* count using appropriate agar and incubated at 37^0c for 24hours

2.4 Data Analysis

All the result was subjected to statistical analysis and the mean were separated using statistical package (SPSS 21.00). All analysis were reported as means of three replicates and subjected to standard deviation and variance (ANOVA)

III. RESULT

3.1 Result of the Sensory Properties of the Chocolate Samples

Table 1 shows the sensory properties of the chocolate samples evaluated. This includes chocolaty flavor, color, texture, taste, mouth feel and overall acceptability The chocolaty flavor, color, texture, taste, mouth feel and overall acceptability values ranged from 5.80 to 7.43, 5.73 to 7.35, 5.93 to 7.25, 4.95 to 6.50, 6.02 to 7.28 and 6.20 to 7.67 respectively. There was significant ($P<0.05$) difference among the samples of chocolate in all the sensory attributes evaluated.

3.2 Result of the Microbiological Qualities of the Chocolate Samples

The results for the bacteria viable count, total viable count of *Staphylococcus aureus* and the viable count of *Eschericia coli* are presented in Table 2, 3 and 4 respectively The count ranged from 11.00 to 24.00 x 10^6, 2.0 to 6.0 x $l0^6$ and 3.0 to 4.0 x $l0^6$ cfu/g Organisms present are *Bacillus subtilis, Kliebsiella spp, Staphylococcus aureus* and *Escherichia coli.* The morphological detail of bacteria isolates of chocolate samples produced from different varieties of cocoa beans is presented in Table 5. The colours of the colonies on agar are white, grey-white and creamy white with size ranged from 1-5mm. All colonies are round in shape except isolate Al with irregular shape The isolates were flat, raised while some were slightly raised with smooth edges while isolate Al had rough edges. All isolates were all opaque with wet surfaces. The morphological and microscopical characteristic of fungal isolates is presented in Table 6. The microscopy view shows that the organisms are cotton- like colonies, black filamentous colonies and green filamentous colonies While the microscopy view shows that the organisms have hyphae with rhizoids, rough conidiophores, smooth walled conidiophores and hyphae without rhizoids.

Table 1: Sensory Properties of Chocolate from New Cocoa Varieties

Sample	Chocolaty flavor	Texture	Taste	Mouth Feel	Color	Over acceptability
TC1	**7.**53 ± 1.46^{a}	7.28 ± 1.61^{a}	6.50 ± 2.33^{a}	7.28 ± 1.71^{a}	7.35 ± 1.75^{a}	7.67 ± 1.54^{a}
TC2	7.43 ± 1.41^{ab}	7.25 ± 1.87^{a}	6.23 ± 2.19^{b}	6.22 ± 0.5^{ab}	7.32 ± 1.59^{a}	7.17 ± 1.72^{ab}
TC5	5.80 ± 2.44^{c}	6.05 ± 187^{b}	5.05 ± 2.75^{c}	6.15 ± 2.48^{b}	5.73 ± 2.41^{d}	6.20 ± 2.02^{c}
TC6	6.28 ± 2.47^{b}	5.93 ± 2.4l^{c}	4.95 ± 2.39^{c}	6.02 ± 2.50^{c}	6.13 ± 2.32^{c}	6.37 ± 2.37^{bc}
TC7	5.85 ± 2.34^{c}	5.67 ± 2.24^{c}	5.05 ± 2.61^{c}	5.95 ± 2.27^{c}	5.90 ± 2.28^{c}	6.47 ± 2.21^{bc}

Values are means ± standard deviation of multiple determinations. Difference letter scripts in the same column indicates statistical difference (P<0 05).

Keys, TC_1, TC_2, TC_5, TC_6, TC_7, (Chocolate produced from Cocoa hybrids TC_1, TC_2, TC_5, TC_6, TC_7)

Table 2: Total Viable Count of Bacteria of Chocolate from new Varieties

Samples	Total viable count ($x10^6$ cfu/g)	Organism
TC_1	11.0 ± 1.0	*Bacillus subtilis, Klebsiella spp*
TC_2	21.0 ± 1.0	*Bacillus subtilis, Staphylococcus aureus*
TC_5	24.0 ± 0.00	*Bacillus subtilts, Staphylococcus aureus*
TC_6	19.0 ± 2.0	*Bacillus subtilis, Escherichia coli, Klebsiella spp*
TC_7	15.0 ± 1.5	*Bacillus subtilis, Escherichia coli, Klebsiella spp*

Keys, TC_1, TC_2, TC_5, TC_6, TC_7, (Chocolate produced from Cocoa hybrids TC_1, TC_2, TC_5, TC_6, TC_7)

Table 3: Total Viable Count of *Staphylococcus aureus*

Samples	Total viable count ($x10^6$ cfu/g)	Organism
TC_1	Nil	Nil
TC_2	2.0 ± 1.0	*Staphylococcus aureus*
TC_5	6.0 ± 0.00	*Staphylococcus aureus*
TC_6	Nil	Nil
TC_7	Nil	Nil

Keys, TC_1, TC_2, TC_5, TC_6, TC_7, (Chocolate produced from Cocoa hybrids TC_1, TC_2, TC_5, TC_6, TC_7)

Table 4: Total Viable Count of *Escherichia coli*

Samples	Total viable count (x106 cfulg)	Organism
TC_1	Nil	Nil
TC_2	Nil	Nil
TC_5	4.0 ± 0.0	*Escherichia coli*
TC_6	3.0 ± 0.0	*Escherichia coli*
TC_7	Nil	Nil

Keys, TC_1, TC_2, TC_5, TC_6, TC_7, (Chocolate produced from Cocoa hybrids TC_1, TC_2, TC_5, TC_6, TC_7.

Table 5: Morphological Characteristic of Bacteria Isolates of Chocolate Samples

Isolate ID	Size (mm)	Shape	Edges	Elevation	Colour	Consistency	Opacity
A1	3-5	Irregular	Rough	Flat	Grey-white	Wet	Opaque
A2	2-4	Round	Smooth	Raised	White	Wet	Opaque
A3	1-2	Round	Smooth	Slightly raise	Creamy white	Wet	Opaque
A4	2-4	Round	Smooth	Flat	White	Wet	Opaque

Table 6: Morphological and Microscopical Characteristic of Isolates of Chocolate Samples

Sample	Macroscopy	Microscopy	Organism
F1	Cotton-like colonies	Hyphae without rhizoids	*Mucor spp*
F2	Black filamentous colonies	Rough conidiophores	*Aspergillus niger*
F3	Green filamentous colonies	Smooth walled conidiophores	*Aspergillus fusarium*
F4	Cotton-like colonies	Hypae without rhizoid	*Rhizopus spp*

IV. DISCUSSION

The sensory properties for the experimental chocolate samples produced from different varieties of new Nigeria cocoa bean is presented in Table 5 Sensory analysis is a scientific discipline that applies the use of human senses for the purpose of evaluating consumer products [9]. Amino acids generated during fermentation by proteolysis of vicilin storage proteins, by natural seed-occurring aspartic proteinases and carboxipeptidases appear to be the precursors of chocolate flavor [10] after reacting with reducing sugars during roasting in a Maillard reaction [11]. The chocolate produced from TC_1 was judged best by the panelists followed by TC_2, when sample TC_5 and TC_7 were least judged in terms of chocolaty flavor. Texture of chocolate sample is the reflection of hardness of the sample which reflects the• nature of the coarseness and particle size of the raw materials used, this corroborate with the findings of [12]. The texture of the chocolate samples showed significant difference ($p<0.05$) with the sample produced with TC_I having the highest mean score, followed by TC_2 while the least judged chocolate samples was recorded form TC_7. In terms of taste, the most preferred chocolate sample was from T_1 while the least preferred was from TC_6 It was observed that there were significant difference ($p<0.05$) among al1 the samples evaluated. The taste of food is an important sensory attributes of foods because of its influence on acceptability [13]. Generally, the same trend that was observed for other sensory parameters was observed in terms of mouth feel and color of the chocolate samples Color is an important parameter m judging food products and **it** doesn't only reflect the suitable raw materials used for the preparation but also provides information about the formulation and quality of the product [9], [14] reported that the products from cocoa bean fermentation (ethanol, acetate and lactate) plus heat directly affect the bean components, causing important biochemical changes leading to the development of typical chocolate color, flavor, and aroma precursors. The overall acceptance expresses how the consumers or panelists accept the

product generally [12]. It is inclusive of all sensory attributes. However, the overall acceptability expresses how the panelists or consumers accept the product generally. The overall acceptability of the chocolate samples showed slight significant ($p<0.05$) with sample TC_1 being judged best and most accepted while TC_5 was least accepted. Sample TC_2 was rated next to TC_1 It could be concluded from the overall acceptability scores that different varieties of cocoa utilized in the production of chocolate in this present study could be utilized in chocolate production without affecting its sensory properties since non score below five which is minimum acceptance score on a 9-point hedonic scale, this result is similar to the findings of [13]. The result for the microbial enumeration of chocolate samples showed that the chocolate produced from different varieties cocoa beans shows a high level of total viable bacteria count. This might be attributed to increase in the microbial population during fermentation process. [15] reported that the cocoa bean pulp is rich in fermentable sugars such as glucose, fructose and sucrose, and has a low pH of 3.0 - 3.5, mainly due to the presence of citric acid These conditions select for the initial growth of yeasts and lactic acid bacteria from the results obtained in the present study, it was shown that the mean bacterial counts of the chocolate samples obviously exceeded the maximum recommended standards by the International Commission on Microbiological Specification of Foods (ICMSF, 1978 cited by [16]**.** According to this agency, the acceptable limit of mesophilic aerobic bacteria in chocolate related food products should not exceed a recommended maximum **value of 10^3cfu/g**. On the other hand, all the results of the *Staphylococcus aureus* and *Escherichia coli* counts from all the chocolate samples analyzed were within the acceptable limit. Presence of aerobes in such a high count in samples indicates neglect to sanitary measures and indicative of post processing contamination as these organisms will be unable to survive the heat treatment applied during processing. The result was in close agreement with those obtained by [16] for repackaged sweets sold in metropolitan Kano, Nigeria.
The result shows that no *Staphylococcus aureus* chocolate produced from TC_1, TC_6 and TC_7, no *Escherichia coli were* detected in chocolates from TC_1, TC_2 and TC_7 but these organisms were detected in others. However, the counts are considerably high since no microorganism should be recovered in any food meant for human consumption (FAO, 1993) [17]. The presence of coliform indicates fecal contamination and the poor level of hygiene after processing. Coliform (except *E. coil)* do not pose danger for consumers, but are indicators of presence of dangerous and pathogenic species in food and water There is a zero tolerance for coliform presence m foods (ICMSF, 1986 cited by [18], and a count of 4000 is of serious concern [19]. Also, coliforms has been established and recommended by public health authorities worldwide as an indicator of post processing contamination in food [20]. The re-packaging materials are also a possible source of contamination (Frazier and Westhoff, 1978 cited by [16] because they are ordinarily wrapped and the wrappers are not subjected to any bacteriostatic or fungistatic treatment.

The cultural and morphological characterization of the microbial isolates recovered from the chocolate samples analyzed in this study indicated the presence of *Staphylococcus aureus, Bacillus sp, Escherichia coli Kliebsiella spp, Aspergillussp, Mucorsp, Rhizopus spp* the predominant contaminants. Particularly important are the *Staphlococcus aureus* and *Bacillus sp.* These are known causative agents of food poisoning• and intoxication (Adams and Moss, 1995). The presence of these bacteria may be due to the unhygienic environmental conditions and poor handling. Various researchers have reported that the presence of *Staphylococcus aureus* in food is an indication of environmental and human contamination [22], [23], [24]**.** According to [19], the presence of *Staphlococcus aureus* might be attributed to contamination of foods through air, mouth and respiratory tract of the handlers. The recovery of *Staphlocbccus aureus* from the chocolate samples examined in this study could be traced to the fact that it is abundant in human body especially as a normal flora of the skin. It is also reported to contribute 40-50% nasal carriers in humans [21], [25]. Of particular importance is the ability of *Staphiococcus aureus* to elaborate enterotoxins in foods, which are dangerous to human and other animal health (Wieneke *et al.,* 1993). The occurrence of this bacterium in the present study could be due to the contamination from many sources, which may include soil, air and water. The organism might have come in during processing; an observation that goes to support Pederson, (1979) cited by [16] who reported that spores of molds and *Bacillus* abound in air and water. The fungi isolated in this study are mostly contaminants which are *Rhizopus spp, Aspergillus fusarium, Aspergillus niger* and *Mucor spp.* The surrounding air, packaging materials and the personnel concerned with the packaging processes could all serve as sources of these contaminants. This agrees with [23] as well as [26] who isolated these organisms and reported that they could be contaminants from air materials used in processing. The isolation of these organisms gives serious cause for concern because *Aspergillus* species is specifically known to produce mycotoxins [21], which cause food intoxication in man and other animals. The presence of fungi suggests the presence of fermentative organisms [27] even though no yeast was isolated. This is because of substances as food because of the large numbers of enzymes it produces. It is capable of causing spoilage of food products containing high sugar concentration [28]. Thus, the presence of *Aspergillu*s species in chocolate samples examined in the present study could result in the production of toxic substances (mycotoxins), which could lead to health hazards for the consumer [28] cited by [16]. This result obtained for microbial count in this study was in close agreement with those obtained by [29]

for chocolate-coated Chinese chest nut for commercial use. However, the non-presence of these organisms in some samples of chocolate is an evident of stability of the chocolate.

V. CONCLUSION

In general, all chocolate samples were judged acceptable by panelist with mean score above 5 which is the Minimum acceptable score on a 9-point hedonic scale while samples TC1 and TC2 were the most acceptable in term of consumer acceptability. Absence of certain organism in microbial identification of samples TC1 and TC7 is an evidence of Stability of the Chocolate samples.

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