

Effect of Inclusion of Cocoa Powder on Antioxidant, Mineral, Microbial and Sensory Properties of Beske, a Tofu-Like Cheese, Made From Soyabean Milk

Ajewole, A.O¹., Raji, M.O¹., Agbola, O.O¹ and Babarinde, Y.A²

1*Value Addition Division, Cocoa Research Institute of Nigeria, PMB, 5244,

2* Food Technology Department, University of Ibadan, Ibadan, Nigeria

Dugbe, Ibadan, Nigeria.

Corresponding Author: Mr. Ajewole, Abiodun Oluwasola, Research Scientist, Value Addition Research, Cocoa Research Institute of Nigeria, Ibadan, Nigeria.

E-mail: abiodunoajewole@crin.gov.ng, presifood2002@yahoo.com, Tel: +234 (0) 8028585681

ORCID No.: 0000-0002-7240-9396

Abstract: 'Choco-beske' was made by incorporating cocoa powder (CoP) at 0- 7.5% into soya milk (SoM). The antioxidants level, DPPH, radical scavenging, vitamin C, mineral contents and microbiological analysis of the produced soya-cheese were carried out using standard procedures. Sensory evaluation of the choco-beske was done using taste panelists comprising 25 female and 15 male members of staff of the Cocoa Research Institute of Nigeria who are regular consumers of beske. The radical scavenging activities of the choco-soy cheese, FRSA and the Vitamin C contents increased from 51.50- 68.14%, 20.78- 37.14% and from 11.69- 21.43mg/100g respectively as the cocoa powder addition increased. The calcium, Iron and magnesium contents of the beske varied from 56.25– 83.01, 10.13– 24.07 and 35.10- 60.96 mg/100g respectively. Cocoa powder in the 'beske' had consequent reduction impact on bacteria colonies count, ranging from 3.02×10^5 to 2.25×10^4 as inclusion increased. Mould growth was completely suppressed in the choco-soy 'beske' stored for six weeks except for the control (no cocoa powder). The result of consumer acceptability test showed that FCoB (5% : 95%) and SFCoB (7.5% :92.5%) had higher scores and were mostly preferred by panelists aside ZCoB (100% choco-beske), the control sample.

Keywords: Cocoa powder, 'Beske', Antioxidants, Storage, Sensory properties

INTRODUCTION

Soy-cheese, otherwise known as 'beske' in the western parts of Nigeria, is a nutritious meat analogue, prepared by curdling the milk extract from soyabean (Abiodun *et al.*, 2023). This is similar to 'tofu', a fermented soybean curd, which is traditionally important and popular in Asia (Han, 2001). Soybean (*Glycine max* L), a leguminous plant, is a rich source of good quality protein. It provides several therapeutic benefits because of its essential amino acids in the amounts needed for human health. In tropics where animal protein is scarce and expensive, products made from soybean are rich plant protein. It could be a supplement to cereal foods where lysine is limiting (Crittenden and Bennett, 2005).

Cocoa bean is one of the best known sources of dietary polyphenols; the total polyphenol content of the dried fat-free-mass of fresh cocoa beans is around 15–20% and of fermented beans approximately 5% (Wollgast and Anklam 2000). The polyphenol content in cocoa beans depends on their origin and processing, as they are subject to a combination of fermentation and drying treatments, followed by alkalisation and roasting, which affects the polyphenol content and consequently the end product quality (Hii *et al.* 2009).

The challenge of poor presentation, high perishability and poor preservation (Adetunji and Salawu, 2008) necessitated the idea of incorporating cocoa powder in beske production. This will contribute significantly to improving its nutritional profile, keeping quality and increased acceptance in the future. Thus, this research effort is geared towards assessment of the antioxidant, mineral, sensory properties and storage stability of cubic shaped processed choco-soy beske.

Materials and methods

Well dried and clean soyabeans was obtained from Oja Oba market in Ibadan while cocoa powder was supplied by the marketing department of CRIN, Ibadan, Nigeria. Alum was obtained from open market in Ibadan and five percent concentration was prepared.

Production of choco-soy-beske (tofu-like cheese)

Choco-soy beske was produced in accordance with the procedure reported by Abiodun A. *et al.*, 2023. 'Beske' were produced from 1000 mL of soyabean milk with cocoa powder added at varying proportions, to create a ratio of (CoP : SoM) 0:100; 2.5:97.5; 5:95;

and 7.5:92.5 (w/w). Alum solution was used as a coagulant. 20 mL of the 5% w/v (5 g in 100 mL distilled water) alum solution was added to each of the milk blends.

Methods of analyses

Antioxidants level (DPPH, FRAS and vitamin C) and minerals content was analyzed according to the official method of analysis described by the Association of Official and Analytical Chemist (AOAC, 2012).

Microbial analysis

Two grammes of truly representative portion of each of the choco-soy-beske samples were aseptically homogenized in 18 mL sterile distilled water. The suspensions obtained were serially diluted until 10^{-5} dilution was obtained for each of the samples. 1 mL of each sample's 10^{-3} dilution was pour-plated with freshly prepared but cooled (45°C) potato dextrose agar (PDA, Oxoid) while same volume of the 10^{-5} dilutions was pour-plated with freshly prepared nutrient agar (NA, Oxoid). The inoculated PDA plates were incubated at room temperature ($30 \pm 2^{\circ}\text{C}$) for 2-5 days, while the NA plates were incubated at 37°C for 24 hours. At the end of the incubation period, the bacteria and fungi (mould) colonies that emerged on the NA and PDA respectively, was counted and their populations (colony forming units, CFU) per gramme of sample were calculated and recorded. The bacteria and mould counts were enumerated in triplicates.

Sensory evaluation

Sensory evaluation was conducted using a 9-point hedonic scale and a semi-trained panel comprising 25 female and 15 male members of Cocoa Research Institute of Nigeria familiar with the consumption of cheese. The panelists evaluated the coded samples for colour, taste, flavour, toughness, and overall acceptability. Each sensory attribute was rated on a 9- point hedonic scale (9 = like extremely and 1 = dislike extremely) (Iwe, 2007).

Statistical Analysis

The data collected was subjected to statistical analysis using SPSS 20; analyses of variance (ANOVA) were used to determine the differences at 5% level of significance. In cases where differences occurred, the means were separated using duncan test.

Results and discussion

Table 1: DPPH, FRSA and Vitamin C content of unripened choco-soy cheese

Sample	DPPH (%)	FRSA (%)	Vit. C (mg/100 g)
ZCoB	51.50 ^d	20.78 ^d	11.69 ^d
TfCoB	64.21 ^c	31.74 ^c	21.43 ^a
FCoB	66.54 ^b	35.35 ^b	18.22 ^b
SfCoB	68.14 ^a	37.14 ^a	16.02 ^c

Values are means of triplicate determination, ^{abcd}Values in the same column bearing different superscripts are significantly different ($p < 0.05$)

Legend: ZCoB (0 : 100), TfCoB (2.5 : 97.5), FCoB (5 : 95), SfCoB (7.5 : 92.5)

Table 1 shows the result of antioxidant capacities of the choco-soy beske. The DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) scavenging effect analysed on the produced beske revealed an increasing trend with the ZCoB having least value. It was observed that the DPPH value increased as the portion of cocoa powder increased in the cheese. Highest value (68.14%) was recorded in SfCoB. The DPPH scavenging effect was significantly different ($p < 0.05$) in all samples. Free radical scavenging activity evaluated as ABTS+ cation scavenging effect [2,2-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid)] also increased across all samples of cocoa-soy beske. Free radical scavenging activity was highest in SfCoB, followed by FCoB, TfCoB and least in ZCoB. Vitamin C content in formulated cocoa-soy cheese was generally high. According to Ibhaze *et al.*, (2020), milk generally is poor in vitamin C and whilst comparing results with United States daily recommended allowance (US RDA), vitamin C in all the samples outweighs

4 percent per serving prescribed. This shows the impact of cocoa powder inclusion in the formulated 'beske'. This revealed the functional attribute of choco-soy beske as a carrier for antioxidants, thereby promoting healthy population and nutrition management through diets.

The mineral elements analysed as shown in Table 2, revealed significant differences ($p < 0.05$) in calcium, iron and magnesium contents of the choco-soy beske samples. ZCoB had the least calcium content of 56.25mg/100g.

Table 2: Calcium, Iron and Magnesium content of choco-soy cheese ('Beske')

Sample	Calcium (mg/100 g)	Iron (mg/100 g)	Magnesium (mg/100 g)
ZCoB	56.25 ^c	10.13 ^d	35.10 ^d
TfCoB	80.06 ^b	20.90 ^c	46.93 ^c
FCoB	81.03 ^b	22.18 ^b	48.92 ^b
SfCoB	83.01 ^a	24.07 ^a	60.96 ^a

Values are means of triplicate, ^{abcd}Values in the same column bearing different superscripts are significantly different ($p < 0.05$). ZCoB (0:100), TfCoB (2.5:97.5), FCoB (5:95), SfCoB (7.5:92.5)

An increasing trend was observed in the mineral content of all the samples as the inclusion level of cocoa powder increased, thus laying credence to the impact of cocoa addition. For iron content, ZCoB had 10.13mg/100g and increased up to 24.07mg/100g with similar trend as observed for magnesium (35.10– 60.96mg/100g). It can be stated that mineral load seen in cocoa-soy beske could be traced to the interaction of cocoa with soy-milk. Lawal and Adedeji (2013), reported low level of mineral in cheese sample obtained and analysed across different locations in Lagos with calcium (10.07, 8.79 and 9.07), Iron (5.63, 4.38 and 5.00) and Magnesium (5.00, 4.65 and 5.12). Similar reports were presented by earlier researchers on the mineral composition of 'warankashi' (Farrow *et al.*, 1999, Hoyen and Koale, 1997, Fasakin and Unokiwedi, 1992). In the light of this, it is necessary to state that mineral content of market sold unripened cheese, 'warankashi', is grossly less and fall short of Food and Drug Administration (FDA) daily requirement (Calcium-1000mg/day, Iron-15mg/day and Magnesium-350mg/day), (info@lenntech.com/rdi.htm). These mineral elements are significant in their food value and necessary for growing children, young and old. Choco-soy beske could be a good supplement in foods deficient of the mineral.

Total bacteria and fungi counts were presented on Table 3. Total bacteria count was higher in ZCoB and keeps decreasing with increased cocoa powder addition. This showed that cocoa powder has the potential to inhibit bacteria growth. Hence, its inclusion in choco-soy beske confers wholesomeness and safety.

Table 3: Total bacteria and mould counts of choco-soy cheese ('Beske')

Sample	Bacteria count (cfu/g)	Mould/yeast count (cfu/g)
ZCoB _f	3.02 x 10 ⁵	5.00 x 10 ²
TfCoB _f	4.15 x 10 ⁴	0
FCoB _f	2.70 x 10 ⁴	0
SfCoB _f	2.25 x 10 ⁴	0

ZCoB ₆	6.00 x 10 ⁴	5.00 x 10 ²
TfCoB ₆	2.60 x 10 ⁴	0
FCoB ₆	2.58 x 10 ⁴	0
SfCoB ₆	2.15 x 10 ⁴	0

Legend: Sample code with subscript f indicates FRESHLY prepared while sample coded with subscript 6 indicates sample STORED at ambient condition for 6 weeks. ZCoB (0:100), TfCoB (2.5:97.5), FCoB (5:95), SfCoB (7.5:92.5)

Total bacteria and fungi counts were presented on Table 3. Total bacteria count was higher in ZCoB and keeps decreasing with increased cocoa powder addition. This showed that cocoa powder has the potential to inhibit bacteria growth. Hence, its inclusion in choco-soy beske confers wholesomeness and safety. Fungi growth was also inhibited in both the fresh and choco-soy cheese stored for six (6) weeks. This result again suggests that cocoa powder contains antifungal substances. The authenticity of this performance cannot be unconnected to combined effect of heat applied during frying and natural active substances present in cocoa powder.

Table 4: Sensory evaluation choco-soy cheese ('Beske')

Sample	Colour	Taste	Flavour	Toughness	Overall acceptability
ZCoB	7.80 ^a	5.35 ^b	6.60 ^a	7.50 ^a	7.55 ^a
TfCoB	6.75 ^{ab}	6.05 ^{ab}	6.70 ^a	7.10 ^a	6.70 ^b
FCoB	6.45 ^{ab}	5.65 ^{ab}	6.95 ^a	6.85 ^a	7.05 ^{ab}
SfCoB	7.30 ^a	6.95 ^a	7.20 ^a	6.35 ^a	7.75 ^a

Values are means of triplicate determination, ^{abcd}Values in the same column bearing different superscripts are significantly different (p<0.05)

Legend: ZCoB (0 : 100), TfCoB (2.5 : 97.5), FCoB (5 : 95), SfCoB (7.5 : 92.5)

Table 4 presents the sensory mean scores of taste panelist who rated the produced cocoa-soy beske. There were significant differences (p<0.05) observed in all the products, implying there was variations in the test products. This variation is partly attributed to varying inclusion levels of cocoa powder in the blends and partly due to the nature of soya milk itself. For colour or appearance, ZCoB (100% soy cheese) as well as SfCoB were rated best followed by TfCoB and FCoB respectively. The cocoa-soy cheese blend, SfCoB, appeared more like meat analogue, 'liver', hence the acceptance. This result was at variance with Aworh and Akinniyi, (1989), and Igyor *et al*, 2006, as substitution results to discolouration (cheese analogue) led to reduced acceptability. In terms of toughness, no significant differences exist between all the samples. It is opined that cocoa inclusion in the blend did not adversely impair the anticipated smoothness, firmness and coherence of produced cocoa-soy cheese. A few respondents remarked on toughness of ZCoB but rarely noticed in samples with varying level of cocoa inclusion. For taste, choco-soy beske with highest cocoa inclusion was rated best, scoring 6.95, while there was no difference compared with ZCoB (100% soya milk), TfCoB (97.5% soya milk) and FCoB (95% soya milk) respectively whereas, flavour across all made cocoa-soy cheese blends were similar (no significant difference). In terms of overall acceptability, SfCoB had the highest rating (7.75), followed by ZCoB, control, with mean score (7.55), and FCoB (7.05). TfCoB, had the lowest score (6.70). This investigation shows consumers' willingness to try out seemingly new products. For all the attributes rated, FCoB and SfCoB were accepted as well as ZCoB.

Conclusion

This study showed there was an increase in antioxidants and mineral level as cocoa inclusion increased. A new product, meat analogue, was produced for the populace. The nutritional value inherent in produced 'beske' by inclusion of cocoa in soy cheese

will improve the well being of the populace from Nigeria and other developing nations. Lastly, cocoa can be utilized in making functional cheese for categories of people regulating their carbohydrate intake. This can be served as a snack food or component of a meal. FCoB and SFCoB beske were acceptable to panelist and therefore recommended.

Declaration of interest

There was no conflict of interest in the course of this research work.

Appreciation

The authors appreciate the Executive Director and CEO, Cocoa Research Institute of Nigeria (CRIN).

References

- Adetunji, V.O. and Salawu, O.T. 2008. West African soft cheese 'wara' processed with *Calotropis procera* and *Carica papaya*: A comparative assessment of nutritional values. *African Journal of Biotechnology*, vol. 7 (18) pp 3360- 62.
- Ajewole, Abiodun O., Aroyeun, S.O and Raji, O.O. 2023. Evaluation of the effect of inclusion of cocoa powder on yield, colour and proximate composition of 'beske'- a tofu-like cheese, made from soyabean milk. *African Journal of Food Science and Technology*, 14 (1), pp 01-04
- AOAC, Official Methods of Analysis. 2012. *Association of Official Analytical Chemists* (17th Edition) Washington D. C., USA.
- Ashaye, O.A., Taiwo, O.O. and Adegoke, G.O. 2006. Effect of local preservative (*Aframonium danielli*) on the chemical and sensory properties of stored warankashi. *African Journal of Agricultural Research*, 1(1): 10-16.
- Aworh, O. C. and Akinniyi, A. 1989. Consumer Acceptability and Microbiological Quality of 'Soy Warankashi' A Nigerian Soft Cheese Made from A Mixture of Soymilk and Cow Milk. *Nigerian Food Journal*, 7:50 – 56.
- Crittenden, R.G. and Bennett, L.E. 2005. Cow'smilk allergy: A complex disorder. *Journal of the American College of Nutrition*, 24: 582- 91
- Farrow, R.P., Kemper, K. and Chin, H.B. 1999. Natural variability in nutrient content of California fruits and vegetables. *Food Technology* 33:52-54.
- Food Drug and Administration. 2020. Recommended daily intakes of various food supplements www.info@lenntech.com/rdi.htm. Accessed August 17, 2021
- Han, B.Z., Rombouts, F.M and Nout, M.J.R. 2001. A chinese soybean fermented food. *International Journal of Food Microbiology*, 65: 1- 10
- Hii, C.L., Law, C.L., Suzannah, S., Misnawi, and Cloke, M. 2009. Polyphenols in cocoa (*Theobroma cacao* L.). *Asian Journal of Food and Agro-Industry* 2(4):702–722.
- Hoyen, T, and Koale, O. 1997. Physical, chemical and biological changes in food caused by thermal processing. In: Lund, D.B. (1999). *Effects of commercial processing on nutrients*. *Food Technology*, 33(2): 33-37.
- Ibhaze, G.A., Oni, O.D. and Onibi, G.E. 2020. Physico-chemical and sensory properties of unripened cheese fortified with food grade Vitamin C. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 14(11): 01-06.
- Igyor, M.A., Igbian, E.K. and Iorbo, C.I. 2006. Effects of soymilk supplement on the yield and Quality of 'Warankashi'. *Nigeria Food Journal* 24 (1), 92-97.
- Iwe, M.O. 2007. Current Trend in Sensory Evaluation of Foods: Nigeria, Enugu, Rojoint Communication Services Limited, pp 136.
- Lawal, A. K. and Adedeji, O. M. 2013. Nutritional and elemental Analysis of Warankashi (Fermented Milk Product) Sold in Lagos Metropolis. *International Research Journal of Biotechnology*, 4(6):112 – 116.
- Wollgast, J. and Anklam, E. 2000. Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International*, 33: 423–44