1	
2	PROXIMATE ANALYSIS OF SOME SELECTED
3	INFANT FORMULA SOLD IN MAKURDI
4	METROPOLIS

6

5

7 Abstract

Infant formula is a synthetic version of mother's milk and belongs to a class of food materials 8 known as dairy substitutes. This study was designed to evaluate the proximate composition in 9 some selected infant formula sold in Makurdi metropolis. Six (6) different brands of infant 10 formula samples were purchased from the major markets, Wadata market and North bank 11 market Makurdi Benue State, North Central Nigeria. The six different brands comprises of 12 three (3) infant milk formulae and three (3) infant cereal formulae. Samples on some Selected 13 14 Infant Formulae were analysed in the laboratory for proximate composition and the data were 15 subjected to Analysis of Variance to rank the significant differences in means. There were 16 significant different (P < 0.05) across the selected infant formulae sample for moisture, ash, crude protein, crude fate, crude fibre and carbohydrates. The result shows that FRISOGOLD 17 significantly (P < 0.05) has the highest moisture content (11.60%) with cowbell having the 18 lowest (2.45%) while the ash content of cowbell was found to be the highest compared to 19 20 other formulae. In conclusion, the commercial baby food samples (MYBOY, NAN and 21 cowbell) have low moisture content which suggest an asset as this prolongs the shelf life and 22 also inhibits microbial activity on these products thereby preventing spoilage.

23

24

25 INTRODUCTION

26 Infant formula is a synthetic version of mother's milk and belongs to a class of food materials 27 known as dairy substitutes. Milk and its products are foodstuffs that have various forms of 28 usage in nature. It contains more than twenty different trace elements including copper, zinc, manganese and iron which are cofactors in many enzymes and participates in many 29 30 physiological functions in mammals [10]. The overview on infant formula reported that 31 breast milk is the best food for infants, as it prevents Diarrhoea and other infant diseases. It 32 should be the sole source of food for the first six (6) months, and only when breast feeding is 33 not sufficient or if the mother is taking a drug that could harm the baby, should infant formula 34 be administered [12].

35 According to [13] infant formula has almost all the major nutrients as the diet that will 36 enhance the growth of the child and more so that these infant formulae are designed to 37 provide the required nutrients as recommended diet intake (RDI) of minerals for infants and 38 toddlers. Infant formula as food supplements has a part to play in the diets of infants that is very important. In the sense that, they supply to the body minerals and vitamins which is 39 40 required in a larger quantity. Since they are primarily derived from animals or plants, they are 41 therefore mostly milk, soya or cereal-based. They almost have all the nutrient requirements 42 that are in breast milk, although it is difficult to produce a formula equal in all respects to breast milk [17]. 43

There are different brands of infant formulae manufactured and mostly used worldwide; therefore the availability depends on the demand of the people. They include Milk based formulae – Cowbell infant formula, SMA gold infant milk, NAN infant milk, My Boy infant milk, Lactogen, Frisolac Gold, Nutriben etc and Cereal based formulae – Cerelac of different ages like 6 months, 8 months, 12 months (Maize, milk & Rice), Friso Gold (wheat base and rice base), Nutrend, Thrive (pediacain), Nutriben (8 cereals and 4 fruits).

UNDER PEER REVIEW

50 The World Health Organization and Food Agriculture Organization have issued some 51 guidelines to produce infant formula commercially, thereby controlling its production. However, reports have shown that various nutritional inadequacies have been cited in some 52 infant formulae. But when infant formula is formulated in accordance with applicable Codex 53 54 Alimentarius standards, it is nutritionally adequate and safe to be a complementary food and 55 a suitable breast milk substitute. [15] reported that baby food composition varied according to 56 region and economic status. More than 70% of lactating mothers do not breast feed their 57 infants exclusively for the recommended periods of six months [15]. As a result infants are subjected to intake of formula milk. The US Foods and Drugs Administration in 2002 stated 58 59 that infant formula milk was not a sterile product [8]. Therefore, this study was designed to 60 evaluate the composition in some selected infant formula sold in Makurdi metropolis.

61

62 Materials and Methods

63 The study area is Otukpo town in Otukpo Local Government Area and located along latitude 64 $7^{\circ}13^{\circ}57^{\circ}N$ and longitude $8^{\circ}05^{\circ}26^{\circ}E$ of Benue State. It is bounded to the North by Apa Local 65 Government, the East by Obi Local Government, and the South by Ohimini Local 66 Government, all of Benue State. The town is strategically located at the intersection of the eastern railway line and the only trunks "A" road linking the Northern parts of the country to 67 the Eastern parts. The town has double maxima rainfall: the major one is around April – July 68 69 and the minor between September – October. The dry dusty harmattan wind blow from the 70 North between November and December. Depending upon a particular year, there are 71 variations of rain output, thus from no rains, isolated rains or fair to heavy rains. The area is 72 underlain by the Agwu shales, a unit of cretaceous sedimentary formation of the Benue 73 valley. The sedimentary rock consists of lateritic soils, clays and shales with occasional thin 74 lenses of sand stone. Besides, the area is endowed with thick forests. The cultural life of the 75 people in Otukpo is linked to farming, trading, small scale soap-making and palm oil 76 industries.

Sample collection 77

78 Six (6) different brands of infant formula samples were purchased from the major markets,

Wadata market and North bank market Makurdi Benue State, North Central Nigeria. The six 79

80 different brands comprises of three (3) infant milk formulae and three (3) infant cereal

81 formulae.

82 The manufacturer's date, expiring dates and NAFDAC numbers are subsumed in Table 1

83

brand		
Milk Brand	manufacturer's date, expiring dates	NAFDAC numbers
Nan	02 2014, 29 02 2016	01 - 0096
My boy	04 2014, 04 2017	01 – 0901
Cowbell	05 11 2014, 05 11 2016	01 – 0558
Cerelac	11 2014, 05 2016	01 - 8379
Nutrend	03 2014, 09	01 - 0672
Friso gold	03 09 201423 08 2016	01 - 7770

Table 1: manufacturer's date, expiring dates and NAFDAC numbers of different milk

[4] 84

Proximate Analysis of the Selected Infant Formulae 85

Determination of Moisture Content 86

The method described by [2] was adopted. A clean crucible was dried to a constant weight in 87 an air oven at 110° C, cooled in a desiccator and weighed (W₁). Two gram (2g) of the sample 88 was accurately weighed into the previously labeled crucible and reweighed (W₂). The 89

90 crucible containing the sample was dried in an oven to a constant weight (W₃). The
91 percentage moisture content was calculated thus:

92 % Moisture Content
$$=\frac{W_2 - W_3}{W_2 - W_1} \times 100$$
-----(1)

93 Determination of Ash Content

The [1] method was used. The porcelain crucible was dried in an oven at 100° c for 10 minutes, cooled in a desiccators and weighed (W₁). Two grams of the sample was placed into the previously weighed porcelain crucible and reweighed (W₂). It was first ignited and then transferred into a furnace, which was then set at 550°C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed cooled in the desiccators and weighed (W₃). The percentage ash content was calculated as:

100 % Ash content =
$$\frac{W_3 - W_1}{W_2 - W_1} \times 100$$
 -----(2)

101 Determination of crude lipid content

102 The lipid content was determined as in the [1]. A clean, dried 500ml round bottom flasks, 103 containing few anti-bumping granules was weighed (w_1) and 300ml of petroleum ether (40-104 60° C) for the extraction was poured into the flask with soxhlet extraction unit. The extractor 105 thimble containing twenty grams was fixed into the soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractor, and cold water circulation was 106 107 put on. The heating mantle was switched on and the heating rate adjusted until the solvent 108 was refluxing at steady rate. Extraction was carried out for six hours. The solvent was recovered and the oil was dried in the oven at 70°C for one hour. The round bottom flask and 109 the oil were cooled and weighed (W₂). The lipid content was calculated thus: 110

111 % crude lipid content =
$$\frac{w_2 - w_1}{w_t \, of \, sample} \times 100$$
-----(3)

112 Determination of crude fibre

113 The method described by [1] was used. Two grams of the sample was weighed out into a round bottom flask, 100 cm³ of 0.25 M sulphuric acid solution was added and the mixture 114 boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The 115 116 insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100 cm³ of 0.31M sodium hydroxide solution was 117 118 added and the mixture boiled again under reflux for 30 minutes and quickly filtered under 119 suction. The insoluble residue was washed with boiling water until it was base free. It was dried to constant weight in the oven at 100° C, cooled in desiccators and weighed (C₁). 120

- 121 The weighed sample (C_1) was then incinerated in a muffle furnace at 550°C for 2 hours,
- 122 cooled in the desiccators and reweighed (C_2) .

123 Calculation

124 The loss in weight on incineration = $C_1 - C_2$. The calculation was carried out thus:

125 % crude fibre
$$=\frac{W_3 - W_1}{W_2 - W_1} \times 100$$
 ------(4)

126 Determination of Nitrogen and Crude Protein

127 Protein Digestion:

The method of [2] was used. Exactly 1.5 grams of defatted sample in an ashless filter paper was dropped into 300 cm³ kjeldahl flask. 25 cm³ conc. H_2SO_4 and 3 g of digesting mixed catalyst (weighed separately into an ashless filter paper) was dropped into the kjeldahl flask. The flask was then transferred to the kjeldahl digestion apparatus. The sample was digested until a clear green color was obtained. The digest was cooled and diluted with 100 cm³ with
distilled water.

134 Distillation of the Digest:

20 cm³ of the diluted digest was measured into a 500 cm³ kjeldahl flask containing anti-135 bumping chips and 40 cm³ of 40 % NaOH was slowly added by the side of the flask. A 250 136 cm³ conical flask containing a mixture of 50 cm³ of 2 % boric acid and 4 drops of mixed 137 138 indicator was used to trap the ammonia liberated. The conical flask and the kjeldahl flask were then placed on the kjeldahl distillation apparatus, with the tubes inserted into the conical 139 flask and the kjeldahl flask. The flask was heated to distill out NH₃ evolved. The distillate 140 141 was collected into the boric acid solution. From the point when the boric acid turned green, 142 10 minutes were allowed for complete distillation of the ammonia present in the digest. The 143 distillate was then titrated with 0.1M HCl.

144 Calculation:

145
$$\% N = \frac{14 \times M \times V_t \times T_v}{Weight of test sample(mg) \times V_a} \times 100$$

146 % Crude protein = % Nitrogen $(N_2) \times 6.38$ [1]

147 Where M = Actual molarity of acid

148 T_v = Titre value of HCl used

149
$$V_t$$
 = Total Volume of diluted digest

150 V_a = Aliquot volume distilled

151 **Determination of Carbohydrate**

152 The total carbohydrate content was determined by difference. The sum of the percentage

moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100 [1].

154 % Total carbohydrate = 100 - (% moisture + % ash content + crude fat +

155 % crude protein + % crude fi).

156 Statistical analyses

157 The results obtained in this study were subjected to analysis of variance (ANOVA) using 158 Statistical Package for Social Science. For multiple comparisons of means across different 159 infant formulae brand, ANOVA was used. In all the case p-values less than 95% confidence 160 level ($\alpha = 0.05$) were considered significantly different using Tukey test.

161 **RESULTS**

162 Proximate Analysis of Selected Infant Formulae

163 Samples on some Selected Infant Formulae were analysed in the laboratory for proximate 164 composition and the data were subjected to Analysis of Variance to rank the significant 165 differences in means. Result of the proximate composition of selected infant food products is 166 presented in Table 2. There were significant different (P<0.05) across the selected infant formulae sample for moisture, ash, crude protein, crude fate, crude fibre and carbohydrates. 167 The result shows that FRISOGOLD significantly (P < 0.05) has the highest moisture content 168 (11.60%) with cowbell having the lowest (2.45%) while the ash content of cowbell was 169 170 found to be the highest compared to other formulae. The protein content of My Boy and 171 cowbell recorded the significantly (P<0.05) the highest (15.48%) and that of FRISOGOLD 172 (11.91%) was lowest. NAN has the highest crude fat content and CERELAC and 173 FRISOGOLD with the lowest (5.8%). The crude fibre of NUTREND was the highest with 174 (1.65%) and no amount of crude fiber was detected in NAN, MY BOY and COWBELL. 175 FRISOGOLD has the highest carbohydrate content of (70.34%) with Cowbell having the 176 lowest of (47.12%).

SAMPLE	%Moistur	% Ash	% Crude	% Crude	% Crude	%
	e Content	Content	Protein	Fat	Fibre	Carbohydrate
						S
NUTREN	7.37±0.0 ^c	2.15±0.02	14.29±0.01	4.60±0.02	1.65±0.02	69.96±0.02 ^c
D		b	с	d	a	
NAN	3.55±0.01	0.75±0.01	14.00±0.02	28.8±0.02	0.00 ± 0.00	52.90±0.03 ^e
	e	f	d	a	d	
COWBEL	$2.47{\pm}0.02^{\rm f}$	7.05±0.01	15.48±0.01	27.9±0.01	0.00±0.00	47.13±0.01 ^f
L		a	a	b	d	
CERELAC	8.16±0.07	1.95±0.02	14.89±0.02	4.50±0.03	0.40±0.01	70.13±0.02 ^b
	b	c	b	d	c	
MY BOY	4.90±0.14	1.30±0.03	15.48±0.01	23.2±0.02	0.00 ± 0.00	55.24±0.02 ^d
	d	d	a	c	d	
FRISO	11.7±0.07	1.15 ± 0.02	11.91 ± 0.03	4.50 ± 0.01	0.50±0.02	70.36±0.03 ^a
P value	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

Table 2: Proximate Analysis of the Selected Infant Formulae.

180 DISCUSSION

178

179

The difference among proximate analysis of the infant formulae brand can be attributed to the different sources of raw materials processed to manufacture the different brands [16]. The highest value recorded by FRISOGOLD for moisture content was higher than those reported in literature for baby food samples 3.7- 4.9% [9], 3.75- 9.32% [6] and 3.0- 4.60% [20], respectively. The lowest moisture content of 2.45% was found in Cowbell. The moisture content values from FRISOGOLD, CERELAC and Nutrend are all within the standard value

of 5-10% recommended by the Standard Organization of Nigeria [14]. The low moisture of 187 188 MYBOY, NAN and cowbell could be of advantage since it has been noted that low moisture content remains an asset to shelf life and preservation of food nutrients whereas a higher 189 190 moisture content could lead to spoilage through increase in microbial action [17]. The very low moisture contents suggest that 'when properly packaged and stored even under ambient 191 192 conditions, these samples would have long shelf life. Samples NAN, CERELAC, FRISOGOLD, NUTREND and MYBOY were all within the maximum standard value of 193 194 2.0% [14] except for samples cowbell which were higher than the standard range. In similar 195 studies, values of ash content ranged in millet/sovbeans based baby foods from 1.70-2.30% 196 [6] and cereal based baby foods ranged from 2.06 to 2.60% [9]. These were slightly higher 197 than the values obtained in this studies with the exception of cowbell but not far from the 198 maximum recommended standard value [14]. The low ash content in this study might be 199 attributed to the effect of fortification and loss of organic matter during processing. The crude 200 protein values obtained was lower than the values of 15.9-16.9% from other studies [6] but 201 fell within the range of 10.5-15.0% [9] and 14.4-18.2% [20]. The crude protein values of this 202 work was within the range when compared to other works and also falls within the minimum 203 range of 14-17% recommended by [14]. Protein is important for babies in their growth, so 204 increase in the protein content of this food is needed to optimise nutritional values derived 205 from their intake. It is very important that a child gets enough protein in their daily diet. They 206 are the building blocks of body tissue and can also serve as a fuel source [5]. Protein can be 207 found in all cells of the body and is the major structural component of all cells in the body, 208 especially muscle. They are complex combinations of smaller chemical compounds called 209 amino acids which are used as precursors to nucleic acid, co-enzymes, hormones, immune 210 response, cellular repair, and other molecules essential for life. Additionally, protein is 211 needed to form blood cells. Protein is needed by everyone to maintain and repair the body,

UNDER PEER REVIEW

212 and it is especially important for babies and young children because protein supports growth 213 and development. Protein is important for babies because walking requires protein to power muscles, and brain cells need this nutrient to learn speech and language skills. Healthy 1- to 214 215 3-year-olds need 0.55 grams of protein per pound daily, which means the average child 216 should get 16 grams of protein each day [18, 19]. The low protein content observed for 217 FRISOGOLD calls for improvement on these foods by increasing the amount of protein or 218 use of protein supplements or pairing them with other suitable protein source. The crude fibre 219 content in the different brands of infant baby foods was less than 1.65%. These values are 220 lower to the values reported for soy/millet based baby foods 3.30- 5.0% [6]. However they do 221 not compare with other reported values for cereal based baby foods 9.29-10.8% [9] which is 222 higher and exceeds the standard maximum value of 5% based on [14] recommendations. The 223 low fibre content in this study may be due to the fact that dehulled raw materials were used in 224 the formulation. Low fibre influences nutrient availability positively while high fibre lowers 225 plasma cholesterol levels [14]. NAN, Cowbell and My Boy were not detected, this is due to 226 milk dissolution properties and might not needed in the food of infants below six months. 227 The values for crude lipid content in the selected baby foods are ranged from 4.50% in 228 CERELAC and FRISOGOLD through 28.8% in NAN. The values reported for NUTREND, 229 CERELAC and COWBELL, however, do not compare to higher values of 7.0-9.0% [13] and 15.6% -17.7% [9] but lower than the values observed for CERELAC, FRISOGOLD and 230 231 NAN in this study. The recommended standard value for crude lipid fat content for 232 commercial baby foods is a maximum of 10.0% [14] and values obtained for NUTREND, 233 CERELAC and COWBELL from this work do not exceed the recommended standard value. 234 However an appropriate inclusion of essential fatty acids in infants and children's diet is 235 vital, because it does not only increase energy density and ensure proper neural development, 236 but also serves as a transport vehicle for fat soluble vitamins [4]. The carbohydrate content of 237 FRISOGOLD, NUTREND and CERELAC baby food samples evaluated in this work were 238 all above the recommended standard values of 60% minimum. However, these values are 239 higher than that of crude protein and also when compared to values from reported literature 240 67.5-68.75% [6]; 67.95-68.40% [9]. The recommended standard value for carbohydrate 241 content is a minimum of 60% [14] and values from this work suggest that the samples of 242 FRISOGOLD, NUTREND and CERELAC baby foods evaluated contains a sufficient 243 amount of carbohydrate. Carbohydrates are important in infant and children's diet as it 244 provides energy. [4] recommended that foods fed to infants and children should be energy 245 dense ones. This, according to the recommendation is necessary because adequate energy 246 fuels child's metabolism, support growth, keeps their brain and nervous system working and 247 maintains overall health whereas low energy foods tend to limit total energy intake and the 248 utilization of other nutrients and functions as mentioned above [18].

249 CONCLUSIONS

250 The proximate composition and levels of trace metals in purposively six brands of infant milk 251 formula aged 0-6 months sold in Nigeria were determined using Atomic absorption 252 spectrophotometer techniques and they showed significant differences across different 253 brands. Also, the commercial baby food samples (MYBOY, NAN and cowbell) have low 254 moisture content which suggest an asset as this prolongs the shelf life and also inhibits 255 microbial activity on these products thereby preventing spoilage. Commercial baby food 256 good are good source of energy and other mineral elements but cannot be relied on as the sole 257 source of complete nutrient intake needed daily by its consumers since they were all low in 258 protein and fiber. These baby foods have to be paired with other protein of choice to get the 259 full nutrient value expected.

260

261 **REFERENCES**

262	1.	AOAC (1990). Official Methods of Analysis. 15th Edn., Association of Official
263		Analytical Chemists, Washington, D.C. USA, pp. 200-210.
264	2.	AOAC (2006). Official method of analysis (18 th edition). Association of Official
265		Analytical Chemist, Washington D.C. U.S.A.
266	3.	FAO/WHO. (2011). Safety evaluation of certain food additives and contaminants:
267		Prepared by the seventy-third meeting of the Joint FAO/WHO Expert Committee on
268		Food Additives (JECFA). WHO Food Additives Series. 64: 381-497.
269	4.	FAO/WHO.(1998). Preparation and Use of Food Based Dietary Guidelines. Report
270		ofJoint
271		FAO/WHO Consultation. WHO Technical Report series 880, Geneva.
272	5.	Food and Nutrition Board. (2005). Dietary Reference Intakes for Energy,
273		Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids
274		(Macronutrients). The National Academic Press, Washington, D.C.
275	6.	Iombor, T.T., Umoh, E.J. and Olakumi.E.(2009). Proximate Composition and
276		Organoleptic Properties of Complementary Food Formulated from Millet, Soy
277		beans. Pakistan Journal of Nutrition, 8, 1676-1679.
278	7.	Joint FAO/WHO Expert Committee on Food Additives. Tolerable or acceptable daily
279		intakes, other toxicological information and information on specifications. In:
280		Summary Report of the Seventy-Fourth Meeting of JECFA. 2011: 3-9.
281		
282	8.	Lanphear, B., Dietrich, K., Auinger, P. and Christopher, C. (2008). Cognitive deficits
283		associated with blood lead concentrations less than ten micrograms per deciliter in US
284		children and adolescents. Public Health Reports 4: 87-96.
285		
286	9.	Mariam, S. (2005). Nutritive Value of Three Potential Complementary Foods Based
287		on Cereals and Legumes. African Journal of Food and Nutrition Series 5(2): 1-14.
288	10	Mohammed A. A.Gasmalla, khadir E. khadir, Abubakar Musa, Waleed Aboshora and
289		Wei Zhao. (2013). Evaluation of some physiochemical parameters of three
290		commercial milk products: Pakistan Journal of food science. 23 (2):62-65.
291	11.	Nwokolo E. 1996. Lima bean (Phaseolus lunatus). Legume and oilseeds In: Nutrition.
292		E Nwokolo and J Smart (Eds.). London: Chapman and Hall. p:145-155.
293	12	Ojo, R.J., and Olabode, O. S(2013)., Analysis of Heavy Metals and Hydrocyanic Acid
294		in Selected Infant Formula in Abuja, Federal Capital Territory of Nigeria., Scholars
295		Academic Journal of Biosciences.,1(6):318-325.
296	13.	Oskarsson, A(1998)., Risk Assessment in Relation to Neonatal Metal Exposure,
297		Anlyst, 123(1):19-23.
298	14.	SON. (1988). Standard for Foods for Infants and Children: Infant Formula.
299	1.5	NigerianIndustrial Standard, Nigeria.
300	15.	Sullivan J.I. (2008). Cognitive development: Breast milk benefit Vs infant formula
301	16	hazard. Archives of General Psychiatry. 65 (12): 1458-1459.
302	10.	Tai, S., Shao, F., Chia, Y. and Wen, I. (2013). Investigation of the nitrate and nitrite content in mills and mills neuronal of Food and Drugs Analysis 21
303		content in milk and milk powder in Taiwan. <i>Journal of Food and Drugs Analysis</i> 21 (1): 72-70
304 205		(1): 73-79.
305		

306	17. Temple, V.J., Badamosi, E.J., Ladeji, O. and Solomon, M. (1996). Proximate
307	Chemical Composition of Three Locally Formulated Complementary Foods. West
308	African Journal of Biological Science, 5, 134 – 143.
309	18. W.H.O. (2008). Strengthening action to improve feeding of infants And young
310	children 6-23 months of age in nutrition and child health Programmes. Report of
311	Proceedings, Geneva, Switzerland.
312	19. W.H.O. (2009). Infant and young child feeding. Model chapter for textbooks for
313	medical
314	students and allied health professionals. Geneva, Switzerland: WHO Press, World
315	Health Organization.
316	20. William, A., Masters, J. and Kuwornuand, D. S. (2011). Improving Child Nutrition
317	throughQuality Certification of Infant Foods: Scoping Study for a Randomized Trial
318	in Ghana.pp. 15-16.