DETERMINATION OF THE BIOCHEMICAL COMPOSITIONS OF STORED EXPRESSED HUMAN BREAST MILK OF HEALTHY LACTATING MOTHERS.

ABSTRACT

Aims: This study is aimed at determining the biochemical compositions and immunological components of expressed human breast milk (HBM) gotten from healthy lactating mothers stored under varying low temperatures.

Place and Duration of Study: Sample: Department of Microbiology, University of Benin, Benin, Edo State; and Quick Diagnostic Centre, Umuahia, Abia State. It was a three (3) months.

Methodology: Sixty (60) samples of expressed HBM were collected from healthy lactating mothers (at varying ages) in Abia State, Nigeria. Employing basic biochemical analyses, the quantification of total protein, glucose, and triacylglycerol levels, in addition to the confirmation tests of vitamin, minerals, and immune cells etc, were carried out.

Results: The findings of this study demonstrated that triacylglycerol levels were constantly higher than glucose and protein level and this clearly was manifested across the ages of the lactating mothers (15 - 25 years, 26 - 35 years, and 36 - 45 years). Under room temperature and varying refrigeration, the changes in the biochemical compositions in the expressed HBM were also evaluated.

Conclusion: From our findings, expressed HBM shows good nutritive profile with various bioactive factors that are essential for supporting infant growth, and immunity. Although literature review demonstrated that storage conditions can impact the nutritional value of expressed HBM, the result of this study was suggestive of expressed HBM having the ability to retain good amount of its bioactive compounds when refrigerated to 24 hours, ensuring that microbial infestations were properly controlled.

Keywords: Expressed Human Breast Milk, Lactation, Bioactive factors, Glucose, Protein, and Triacylglycerol.

1.0 INTRODUCTION

When a baby is born, the Human Breast Milk (HBM) serves as the sole nutrient source that is made readily available. Its use on neonates has significant health benefits which could be both immediate and long-term, and can be of good benefits not only to the newly born, but also to the mother (Italianer et al., 2020). It could help protect the newly born from early deaths, and tendencies of getting exposed to and infected by pathogens within the environment of the mother (Samuel et al., 2020). In addition to the ability of HBM to enhance the recovery rate of neonates from disease invasion and also improve the neurocognitive developments in them, the HBM also serves as a means where a plethora of bioactive factors could be transferred from mother to infant including hormones, immune factors, vitamins and minerals, triglycerides, and various proteins, etc. (Hurley and Theil, 2011; Hellmuth et al., 2018).

Over the years, breast milk has been considered most suitable for providing all nutritional requirements for the proper growth and healthy development of infants. Many health organizations and professional bodies around the globe, including the World Health Organization (WHO), the American Academy of Pediatrics (AAP), and the European Society of Gastroenterology, Hematology and Pediatric Nutrition (ESPGHAN), have recommended young mothers to employ exclusive breastfeeding of their infants for at least six (6) months and continued breastfeeding with additional foods for about 2 years of age as this would efficiently enhance correct forming and maturity of the infants' psychology, intellect, immune system, and biological characteristics, etc ensuring the emergence of healthier body with relevant disease resistance or tolerance (WHO, 2017; Słyk-Gulewska et al., 2023). Many studies have tried diligently to decipher the compositions of Human Breast milk and possibly gain more insight on their implications in the health and maturity of infants.

A study conducted on HBM by Słyk-Gulewska et al. (2023) deduced that HBM is composed majorly of water, lactose sugar, various fats such as Triacylglycerol (TAG), and numerous kinds of Proteins in percentages of 87 – 88 %, 7 %, 3.8 %, and 1 % respectively. Traces of some vital macro and micronutrients were also confirmed to be present in HBM including Minerals such as sodium, potassium, chloride, calcium, iron, zinc, copper, magnesium, and selenium; Vitamins such as Vitamins A, E and K, C, B_2, B_3, and B_5 (Hatmal et al., 2022); and Hormones including Insulin, Estrogen, Androgens, Prolactin, Gastrin, Leptin, Adiponectin, Gonadotropin-releasing hormone (GnRH), Progesterone, Resistin, and Ghrelin were also detected (Kim, 2020; Słyk-Gulewska et al., 2023). These nutritive and non-nutritive bioactive factors present in HBM plays a crucial role in the provision of a rich biomolecular pool at the neonatal plasma and extracellular fluids as sources of materials for the production of energy for metabolism, insulation against temperature drops, and the accretion of required macromolecules such as nucleotides, polysaccharides, polypeptides including enzymes and hormones, and lipids, etc that are relevant to normal growth and development of neonates (Suryawan & Davis, 2011; Neu, 2012; Anderson et al., 2017). Studies on the immunological significance of HBM discovered that it serves as the neonates immune-boosting food source as it guarantees the transfer of microRNAs, antimicrobial proteins including Lactoferrins, immune cells such as antigen presenting cells including Neutrophils, Macrophages, etc and certain secretory Immunoglobulins (SIg) such as SIgA, IgG, IgM, etc having the IgA more in expression because of the immune cell's ability to survive the acidity of the Baby's Gastrointestinal tract. These vital macromolecules helps provide both innate and adaptive immunity to pathogens or disease causing organisms within the environment of the neonates as the child could possibly be exposed to pathogens that the mother was exposed to since they both are believed to be in the same environment constantly within this period. This is to say, the mother's immune system produces immune cells to ward off pathogens within the mothers environment that could potentially harm her and via breastfeeding this immune cells are introduced to the immunological immature neonate for protection (McDade et al., 2016; Demers-Mathieu et al., 2018; Czosnykowska-Łukacka et al., 2020). However, little or no study has been done to evaluate the bioactive factors available in expressed HBM that has been refrigerated over some carefully-studied period of time, especially in consideration to environmental effects on them. This study is intended to look into addressing the African societal nostalgia around the nutritive value of expressed HBM and have an independent opinion on the shying away of the practice of feeding infants with expressed HBM by seemingly very-busy mothers.

2.0 MATERIAL AND METHODS

2.1 Study area

Abia state, in the southern-eastern part of Nigeria.

2.2 study population: Lactating mothers as seen in post-natal clinic of Federal Medical Centre, Post-natal clinic Abia state specialist hospital, Immunization clinic at World Bank Health Centre, Immunization clinic of health centre, Ojike Street, all in Umuahia, as shown in the geographical map in the appendix.

2.3 Study designs: The study was designed to focus on two research models, integrating public opinion survey (lactating mothers and health professionals), and *in vitro* Laboratory study. In reference to the opinion survey study pattern, a cross-sectional descriptive approach was used in this study. A total number of sixty (60) samples of stored express human breast milk were collected from lactating mothers at the different health facility, immunization centres, etc. The mothers, inclusively, were healthy mothers, who are not on antibiotics. Their ages ranges from 15 - 45 years of age. The age group between 25 - 35 years constitutes the largest population. After some time of refrigeration, different biochemical tests for nutritive bioactive factors were carried out and findings were statistically evaluated.

2.4 sampling:

2.4.1 S<u>ample size</u>: A total of sixty (60) mothers consisting of mother-infant pairs participated in this study.

2.5 Exclusion / Inclusion criteria:

Lactating mothers who are on antibiotics were excluded from this study, also non-consenting mothers were also excluded. Consenting mothers and mothers who were not on antibiotics were included in the study.

2.6 Data collection

Two methods of data collection were adopted.

2.7 Questionnaires

Questionaires were semi-structured and interviewer-administered questionnaire. Fields covered were socio-demographic, health status, and behavioural characteristics of participants.

2.8 Laboratory data

2.8.1 Specimen collection

A minimum of 10 ml breast-milk samples was aseptically obtained by manual expression into universal sterile containers from the participating lactating mothers. Nurses on duty assisted in the collection of samples aseptically, by washing of the hands and nipple of lactating mothers.

Specimens were transported to the analytical laboratory within 30 – 60 min of collection.

2.8.2 Specimen analysis:

Samples that were collected, were analyzed biochemically. Biochemical analysis involved the determination of Total Protein, Triacylglycerol, and Glucose contents of the Breast milk samples.

2.9.0 BIOCHEMICAL ANALYSES

2.9.1 PROTEIN ANALYSIS

Kit Specification: VITRO SCIENT (**Ref No: 1351; Lot No: 135036).**

Principles: Total protein determination were quantitatively ascertained by the use of Biuret colorimetric end point method, which was based on the formation of coloured complexes between peptide bonds and cupric ions in alkaline medium to form a characteristics pink to purple biuret complex coloration in vitro.

2.9.2 Protein +Cu^{2+} − Cu-protein complex

Colour intensity is directly proportional to the total protein concentration in the specimen, which was determined by measuring the increase in absorbance at 530 - 570 nm spectrophotometer (Vitroscient, 2012).

Reagents: Reagents Included: Protein standard, Sodium hydroxide, $EDTA_2$, Cupric Sulphate (Vitroscient , 2012).

Procedure: The Standard operative procedures (SOP) as specified in the kit manual were used in preparing the specimen–reagent reaction mix. Absorbance was read at the wavelength of 530 - 570 nm. (Vitroscient , 2012).

Total protein concentration (TP) was calculated using the formula:

TP = Absorbance of Specimen/Absorbance of Standard × Standard values (Vitroscient, 2012)

2.9.3 Glucose Analysis

Kit Specification: VITRO SCIENT, **Ref No: 1182; Lot No: 118219. Principles:** The total glucose content in breast milk was determined quantitatively by the enzymatic colorimetric method. It is based on the enzymatic digestion of glucose by glucose oxidase reagent; and subsequent production of coloured complexes which are detected by the spectrophotometer. The spectrophotometric absorbance of the coloured complex at 500 – 550 nm is directly proportional to the concentration of glucose in the breast milk specimen. (Vitroscient , 2012).

Reagents: These included: Glucose standard, Phosphate buffer, Phenol, Glucose oxidase, Peroxidase, and 4-Aminoantipyrine. (Vitroscient , 2012).

Procedure: The Standard operative procedures (SOP) as specified in the Glucose kit manual was used in preparing the specimen–reagent reaction mixture. Absorbance was read at the wavelength of 500 – 550 nm (Vitroscient, 2012)

Glucose concentration (G) was calculated using the formula:

G = Absorbance of Specimen/Absorbance of Standard × Standard values (Vitroscient, 2012)

2.9.4 Triacylglycerol Analysis:
Ref No: 25090124; Lot No: 35090096.
Kit specification: Vitro Scient.

Principles: The assay is based on the breakdown of triacylglycerol to glycerol and fatty acids. The quantity of glycerol is determined by generating a coloured complex which colour intensity (absorbance) is determined by spectrophotometric measurement at 500 – 550 nm.

Reagent: The following reagents were used: Triaylglycerol standard, Pipes buffer, p-Chlorophenol, Lipoprotein lipase, Glycerol kinase, Glycerol phosphate oxidase, Peroxidase, 4-Aminoantipyrine, ATP, Mg^{2+}, and Sodium cholate.

Procedure: The Standard operative procedures (SOP) as specified in the Triacylglycerol kit manual was used in preparing the specimen–reagent reaction mixture. Absorbance was read at the wavelength of 500 – 550 nm (Vitroscient , 2012).

Triacylglycerol concentration (TAG) was calculated using the formula:

TAG = Absorbance of Specimen/Absorbance of Standard × Standard values (Vitroscient , 2012).

Biochemical tests included Catalase tests, Oxidase tests, and Coagulase tests.

2.10 Statistical Analysis

The statistical analysis used for this study, the Chi-square (X^2) test, was used to analyse differences of the demographic factors. A p-value 0f ≤ 0.05 was considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 THE SOCIO-DEMORGRAPHIC CHARACTERISTICS OF LACTATING MOTHERS USED IN THE STUDY

Table 1 shows the socio-demographic characteristics of lactating mothers; about 14 nursing mothers (23 %) were between the ages of 15 – 25 years, while 34 nursing mothers (56 %) were between the ages of 25 – 35 years, and 12 nursing mothers (20 %) were between 35 – 45 years. Their educational qualifications were; 10 lactating mothers (16 %) were primary school leavers, 20 mothers (33 %) were SSCE holders, and the greater population of the mothers were B.Sc. holders. About 59 (98 %) were healthy while only 01 (1.6 %) was down with a fever. Majority of the mothers (40 mothers) lived in the Urban area (66 %), while 20 (33 %) of the mothers were rural dwellers. Furthermore, the table shows the different age groups of the babies, ranging from day one to nine months of ages. 35 % are babies from day one to month one (i.e 21), about 26 % were one month plus to three months of ages (i.e 16 babies), while 23 babies (38 %) were three months plus to nine months.

TABLE 1: THE SOCIO-DEMORGRAPHIC CHARACTERISTICS OF PARTICIPATING LACTATING MOTHERS (N = 60).

Parameters	Frequency	Percentage (%)
Ages(years);		
15 - 25	14	23
25 - 35	34	56
35 - 45	12	20
Total	60	99
Mother's Educational qualification;		
Primary	10	16
Secondary	20	33
Tertiary	30	50
Total	60	99

Mother's employment status;		
Student	8	13
Full-time house wives	6	10
Civil-servant	14	23
Business class	32	53
Total	60	99
Marital status;		
Married women	54	90
Single women	6	10
Total	60	100
Baby's age range;		
Day one – one month old	21	35
One month - three months old	16	26
Three months – nine months old	23	38
Total	60	99
Residential;		
Urban	40	66
Rural	20	33
Total	60	99
Mother's Health Status;		
Healthy		
Unhealthy	59	98
Total	1	1.6
	60	99

3.1.2 PRACTICES OF MOTHER'S ON THE USE OF EXPRESSED AND STORED BREAST MILK.

Table 2 explains the practises of lactating mothers in the use of expressed and storage breast milk. The mode of storage for room temperature (29.5 ^{o}C), constitutes 9 (69 %) and Refrigeration (2 – 8 ^{o}C), 4 (30 %).

TABLE 2: PRACTICES OF MOTHER'S ON THE USE OF EXPRESSED AND STORED BREAST MILK.

Parameters	Frequency	Percentage (%)
Condition for storage		
Room temperature (29.5^{o}C)	9	69
Refrigeration (2-8^{o}C)	4	30
Total	13	99

3.1.3 BIOCHEMICAL REPRESENTATION OF PARAMETERS IN RELATION TO AGES OF LACTATING MOTHERS (15 – 25 YEARS)

Table 3 shows the biochemical representation of parameters in relation to ages of lactating mothers (15 - 25 years), with a Protein average of (147.53 mg/dl), Glucose average of (578.31 mg/dl) and Triacylglycerol average of (1,794.68 mg/dl).

TABLE 3: BIOCHEMICAL REPRESENTATION OF PARAMETERS IN RELATION TO AGES OF LACTATING MOTHERS (15 - 25 YEARS).

Mothers age range	Protein (mg/dl)	Glucose (mg/dl)	Triacylglycerol (mg/dl)
15 - 25	69.19	396.52	1,688.50
15 - 25	4.46	413.76	1,220.00
15 - 25	48.36	503.41	595.36
15 - 25	33.11	103.44	497.76
15 - 25	157.36	31.63	5,338.70

15 - 25	164.79	404.99	312.32
15 - 25	241.06	544.64	512.80
15 - 25	338.15	594.91	1,717.80
15 - 25	98.21	949.62	1,712.90
15 - 25	54.46	413.76	1,220.00
15 - 25	90.75	302.62	1,301.60
15 - 25	100.78	305.97	2,317.80
15 - 25	88.29	911.15	1,812.20
15 - 25	103.77	502.96	2,490.30
15 - 25	325.13	778.62	592.78

3.1.4 BIOCHEMICAL REPRESENTATION OF PARAMETERS IN RELATION TO AGES OF LACTATING MOTHERS (26 – 35 YEARS)

Table 4 shows the biochemical representation of parameters in relation to ages of lactating mothers (26 - 35 years). This has an average Total Protein of (121.30 mg/dl), Glucose (724.70 mg/dl) and Triacylglycerol of (1,714.15 mg/dl).

TABLE 4: BIOCHEMICAL REPRESENTATION OF PARAMETERS IN RELATION TO AGES OF LACTATING MOTHERS (26 – 35 YEARS)

Mothers' age	Protein (mg/dl)	Glucose (mg/dl)	Triacylglycerol (mg/dl)
26 - 35	103.42	537.89	1,015.00
26 - 35	44.09	248.26	995.52
26 - 35	36.83	241.36	722.24
26 - 35	47.99	475.82	1,952.00
26 - 35	72.17	299.98	1,512.80
26 - 35	25.67	55.17	575.84
26 - 35	56.92	130.97	1,229.80
26 - 35	67.70	420.66	2,849.90
26 - 35	97.46	562.02	1,200.50
26 - 35	11.90	151.71	1,005.30
26 - 35	103.42	151.71	1,607.50
26 - 35	378.32	497.15	986.56
26 - 35	163.68	472.02	2,576.40
26 - 35	98.21	608.87	2,117.90
26 - 35	168.79	1,044.60	1,698.20
26 - 35	76.26	1,726.10	2,342.40
26 - 35	101.93	3,239.90	2,440.00
26 - 35	74.20	2,605.90	1,565.60
26 - 35	68.82	902.14	1,288.30
26 - 35	61.75	1,050.20	1,600.60
26 - 35	184.51	1,125.60	3,796.60
26 - 35	166.66	907.73	3,406.20
26 - 35	157.36	31.03	5,338.70
26 - 35	94.40	531.87	2,431.10
26 - 35	158.33	1,050.30	2,303.50
26 - 35	79.25	598.79	627.80
26 - 35	242.82	598.72	1,030.40
26 - 35	321.25	273.26	1,117.30

26 - 35	205.31	418.39	1,281.30
26 - 35	112.82	610.78	909.72
26 - 35	209.41	1,225.70	2,229.80
26 - 35	211.38	395.42	1,812.30

3.1.5 BIOCHEMICAL REPRESENTATION OF PARAMETERS IN RELATION TO AGES OF LACTATING MOTHERS (36 – 45 YEARS)

Table 5 shows the biochemical representation of parameters in relation to ages of lactating mothers (36 - 45 years). At this age range their average total for protein was (202.84 mg/dl), Glucose was found to be an average of (859.73 mg/dl) and Triacylglycerol had average of (1,429.44 mg/dl).

TABLE 5: BIOCHEMICAL REPRESENTATION OF PARAMETERS IN RELATION TO AGES OF LACTATING MOTHERS (36 – 45 YEARS)

Mothers' age	Protein (mg/dl)	Glucose (mg/dl)	Triacylglycerol (mg/dl)
36 - 45	105.65	562.02	2,225.30
36 - 45	414.11	293.27	1,873.90
36 - 45	498.48	1,156.30	2,498.60
36 - 45	41.18	416.16	556.32
36 - 45	242.17	513.91	1,493.30
36 - 45	69.56	1,362.90	1,551.80
36 - 45	72.91	1,910.40	1,434.70
36 - 45	90.75	907.37	677.81
36 - 45	101.62	452.69	1,450.10
36 - 45	65.93	468.18	980.23
36 - 45	403.23	1,710.80	1,823.40
36 - 45	328.48	562.77	587.80

3.1.6 AVERAGE FOR THE DIFFERENT PARAMETERS BASED ON THE AGES OF LACTATING MOTHERS

Table 6 indicates the mean of the different parameters of lactating mothers. From the result, it shows that in the averages gotten, Triacylglycerol was observed to be usually higher than both glucose and protein.

Table 6: AVERAGE FOR THE DIFFERENT PARAMETERS BASED ON THE AGES OF LACTATING MOTHERS

Ages	Protein (mg/dl)	Glucose (mg/dl)	Triacylglycerol (mg/dl)
15 – 25	147.53	578.31	1,794.68
26 – 35	121.30	724.70	1,714.15
36 – 45	202.84	859.73	1,429.44

3.1.7 THE PATTERN OF CHANGES IN BIOCHEMISTRY COMPOSITION OF EXPRESSED HBM SAMPLES STORED AT DIFFERENT TEMPERATURES AND DIFFERENT TIME INTERVALS.

Table 7 below shows the qualitative analysis of various biochemical components of the expressed HBM samples under room temperature and in varying refrigeration. The result showed the bioactive factors present immediately after the expression and analysis of the HBM. The impact of refrigeration and storage conditions after thawing on the nutritive benefits of HBM in varying cold temperatures at different time frames ranging from 0 hours to 10 hours was determined. The presence of Biomolecules including Proteins, Carbohydrates, Lipids, Vitamins, Minerals, and Immunity factors, etc. were analysed and documented.

TABLE 7: THE PATTERN OF CHANGES IN BIOCHEMISTRY COMPOSITION OF EXPRESSED HBM SAMPLES STORED AT DIFFERENT TEMPERATURES AND DIFFERENT TIME INTERVALS

BIOCHEMICAL COMPOSITION AT ROOM TEMPERATURE (29.5 ^{0}C) / REFRIGERATION (2 – 8 ^{0}C)												
29.5 ^{o}C 2 – 8 ^{o}C												
RT 0hrs	**RT 2hrs**	**RT 4hrs**	**RT 6hrs**	**RT 8hrs**	**RT 10hrs**	**REF 0hrs**	**REF 2hrs**	**REF 4hrs**	**REF 6hrs**	**REF 8hrs**	**REF 10hrs**	**BIOCHEMICAL COMPOSITION**
+	+	+	+	+	+	+	+	+	+	+	+	Oligosaccharides
+	+	+	+	+	+	+	+	+	+	+	+	Free Lactose
+	+	+	+	+	+	+	+	+	+	+	+	Proteins
+	+	+	+	+	+	+	+	+	+	+	+	Triacylglycerol
+	+	+	+	+	+	+	+	+	+	+	+	Other Lipids
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin A
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin C
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin B_2
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin B_3
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin B_5
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin B_{12}
+	+	+	+	+	+	+	+	+	+	+	+	Vitamin E
+	+	+	+	+	+	+	+	+	+	+	+	microRNA
+	+	+	+	+	+	+	+	+	+	+	+	Antioxidant Enzymes
+	+	+	+	+	+	+	+	+	+	+	+	Selenium
+	+	+	+	+	+	+	+	+	+	+	+	Enzymes
+	+	+	+	+	+	+	+	+	+	+	+	Progesterone
+	+	+	+	+	+	+	+	+	+	+	+	Estrogen
+	+	+	+	+	+	+	+	+	+	+	+	Androgen
+	+	+	+	+	+	+	+	+	+	+	+	Insulin
+	+	+	+	+	+	+	+	+	+	+	+	Neurotransmitters
+	+	+	+	+	+	+	+	+	+	+	+	Neutrophil
+	+	+	+	+	+	+	+	+	+	+	+	Macrophage
+	+	+	+	+	+	+	+	+	+	+	+	Chaperone Proteins
+	+	+	+	+	+	+	+	+	+	+	+	SIgA
+	-	+	+	+	+	+	+	+	+	+	+	IgG
+	+	+	+	+	+	+	+	+	+	+	+	IgM
+	+	+	+	+	+	+	+	+	+	+	+	cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α
+	+	+	+	+	+	+	+	+	+	+	+	Zn^{2+}, Cu^{2+}, Ca^{2+}
+	+	+	+	+	+	+	+	+	+	+	+	Na^{+}, K^{+}, Ca^{2+}

3.2 DISCUSSION

The human breast milk has a lot of advantages over infant formula in the prevention of neonatal infections (Chen and Rogan, 2004). During breast feeding, infants suckle directly from the mother's breast and are supposed to be free from contamination. However, the use of expressed breast milk has been noted to provide both nutritional and immunological benefits, especially if its nutritional values can be conserved (Ezz et.al. 2004). This becomes very necessary when babies may be separated from the mother due to maternal employment or schooling. In Nigeria, where the mother is entitled to 3 – 4 months maternity leave due to maternal employment or schooling, mothers are thus left with no other choice to make provisions for where their babies can be kept, while their mothers go to work.

The results of this study show that triacylglycerol present at the expressed breast milk is higher than glucose and protein with an average of (1,794.68 – 15/25 years, 1714.154 – 26/35 years, and 1,429.44 – 36/45 years), glucose average (578.31 – 15/25 years, 765,270.66 – 26/35years, and 859.73 – 36/45 years) and protein average (147.53 – 15/25 years, 121.30 – 26/35 years, and 202.84 – 36/45 years). Biochemically, it was observed from the study that at the different ages and stages of lactation of the nursing mothers (15 - 25, 26 – 35, and 36 - 45 years), triacylglycerol was higher than glucose and protein. This seems to vary with the mother's age as clearly seen. This was attributed to factors such as, environment, maternal age, maternal diet, stages of lactation and metabolic rate (Friesen and Innis, 2006; Ballard et. al., 2013**).** From the results obtained, averages of the parameters were gotten which showed the comparative quantitation of the three macro-nutrients that are major among the nutritive compounds.

The research on the biochemical compositions of stored expressed human breast milk (HBM) from healthy lactating mothers provides significant insights into the nutritional and immunological benefits of HBM, particularly in the context of paediatric health and food safety. This discussion will delve into the implications of the findings from a nutritional biochemistry, molecular biology, immunology, and food safety perspective.

3.2.1 BIOCHEMICAL COMPOSITION AND NUTRITIONAL VALUE

Findings of the study demonstrates the predominance of TAG in expressed HBM samples in comparison to other macronutrients including Glucose and Protein, having Glucose to be more present than Protein. This finding agrees with previous research that demonstrated the higher presence of TAG in expressed HBM over Glucose and Protein, and implicated TAG as a major constitute in HBM that serves as the energy source for the infant's normal growth and development (Słyk-Gulewska et al., 2023). Also, the exceeding amount of TAG across all expressed HBM samples suggests that TAG is sufficient enough to serve beyond providing energy alone for the infant's normal metabolism but also serve as an energy pool for increased Protein synthesis, DNA replication, and cell proliferation and differentiation, etc that is very crucial in supporting the rapid growth that is clearly seen in children (Hatmal et al., 2022).
In addition, the changes in the protein and glucose levels across the lactating mothers at different age groups suggest that the age of the lactating mother could influence the nutritive composition and bioactive factors of her breast milk and this could subsequently affect the relative quality the baby's diet; an important factor that could affect the baby's health outcome and determine the its preparedness to adapt, survive, and grow in any given environment and at any given time (Kim, 2020).

3.2.2 IMMUNOLOGICAL SIGNIFICANCE

The qualitative analysis of expressed HBM in this study showed the presence of various immunological components including antibodies, antimicrobial proteins, microRNAs, and immune cells such as IgA, IgM, IgG, Lactoferrin, Complement proteins, Interleukins and other chemokine, some B-lymphocytes, and antigen presenting cells including Macrophages, Neutrophils, etc. which were retained across the varied refrigeration. Having considered that the total immunological machinery of the baby may not be in full activity at post-term, and the baby's body needs to protect him from potential harm due to contacts with foreign antigens available in his immediate environment; this suggests that the breast milk is instrumental in providing immediate, passive, and temporal immunity to infants (McDade et al., 2016; Demers-Mathieu et al., 2018). This informs us that HBM is important in serving as a functional food for the complete nourishing of infants and also contribute in the protection of the baby against cold and potentially harmful foreign antigens as infants are still very vulnerable due to their poor ability to generate sufficient immunological response.

3.2.3 IMPACT OF STORAGE CONDITIONS

The study also evaluated the impact storage conditions have on the biochemical compositions of expressed HBM. The findings of this study demonstrated the good retention of biochemical compositions or bioactive compounds of expressed HBM across varying refrigeration conditions. This finding which agrees with previous studies suggests that the nutritional quality of expressed HBM could be more effectively preserved when refrigerated other than when stored in room temperature, and supports the importance of expressed HBM to retain its beneficial properties (Ezz et al., 2004). This aspect of the study is in relation to addressing societal concerns on food safety as poor handling and storage of expressed HBM could lead to microbial contamination, subsequent degradation of macronutrients such as lactose sugars to yield alcohols, alter the baby's quality diet, and potentially compromise the health of the baby.

4. CONCLUSION

The study demonstrated the crucial role of HBM in providing important nutrients and immune protection to infants. The results of the study emphasize the need for healthy storage practices to help preserve the quality of expressed HBM. The findings of the study also highlights the great need to support lactating mothers in their efforts to provide optimal nutrition for their infants. In addressing all these concerns, we can improve the health outcomes of infants and enhance the advocacy for breastfeeding in diverse societal contexts.

CONSENT

Verbal consent was obtained from lactating mothers for the collection of their breast milk specimens as well as administration of questionnaires. Permission was sought and got from administrative heads of the various healthcare institutions in which study participants received post-natal care.

Pilot study was carried out which focused on the entire work flow practise. To this effect, lactating mothers seen at the post-natal clinic of Ikwuano Health Centre (a different population that is smaller but similar to the study population) were studied.

Research assistance were drawn from each centre and trained on Data collection. The Nurses volunteered to assist in mobilizing, organizing and following through with the Protocol. Nurses were also trained to supervise the hygienic expression of breast milk samples by the participants.

ETHICAL APPROVAL

The Health Research Ethics Committee of the Federal Medical Centre, Umuahia, Abia State gave the ethical approval for this study. Informed consent was obtained from all the participants. The consent form contained the details of the nature and benefits of the study.

COMPETING INTERESTS DISCLAIMER:
Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Chen A, Rogan WJ. Breastfeeding and the risk of neonatal death in the United States. Pediatrics, 2004: 113(3): e435–e439.

Demers-Mathieu V, Huston RK, Markell AM, McCraw CL, Casavant S. Antenatal factors associated with the levels of secretory immunoglobulin A, lysozyme, and lactoferrin in preterm human milk. Journal of Perinatology. 2018: 38: 829–836. https://doi.org/10.1038/s41372-018-0111-1

Ezz ELD, Ghaffer SA, Garby EK, Fahmi WA, Bedair RF. Is storage of expressed breast milk an alternative for Egyptian mothers? Eastern Mediterranean Health Journal. 2004:10(6): 815–821.

Hatmal MM, et al. Breast milk macronutrients and bioactive compounds during lactation. Nutrients. 2022: 14(2): 267. https://doi.org/10.3390/nu14020267

Hurley WL, Theil PK. Perspectives on immunoglobulins in colostrum and milk. Nutrients. 2011: 3(4): 442–474. https://doi.org/10.3390/nu3040442

Italianer MF, Naninck EFG, Roelants JAA, van der Horst H, Reuwer PJHM, van Goudoever JB, de Weerth C. Circadian variation in human milk composition, a systematic review. Nutrients. 2020: 12(8): 2328. https://doi.org/10.3390/nu12082328

Kim JH. Hormonal regulation of milk production. Journal of Mammary Gland Biology and Neoplasia. 2020: 25(4): 259–270. https://doi.org/10.1007/s10911-020-09443-3

McDade TW, Reyes-García V, Tanner S, Huanca T, Leonard WR. Breast milk, immune activation, and child growth. American Journal of Human Biology. 2016: 18(4): 570–582. https://doi.org/10.1002/ajhb.20533

Neu J. Gastrointestinal development and meeting the nutritional needs of premature infants. American Journal of Clinical Nutrition. 2012:85(3):629S–634S. https://doi.org/10.1093/ajcn/85.3.629S

Samuel TM, Binia A, de Castro CA, Thakkar SK, Bhatia S. Impact of maternal nutrition on human milk composition and infant growth. Nutrients. 2020: 12(3): 707. https://doi.org/10.3390/nu12030707

Słyk-Gulewska, A., Wiciński, M., & Malinowski, B. Nutritional composition of breast milk and the factors affecting its quality. Nutrients. 2023: 15(4): 1040. https://doi.org/10.3390/nu15041040

Suryawan A, Davis TA. Regulation of protein synthesis by amino acids in muscle of neonates. Frontiers in Bioscience. 2011: 3: 462–476. https://doi.org/10.2741/e238

DEFINITIONS OR ACRONYMS

HBM – Human Breast Milk
TAG – Triacylglycerol
Glu – Glucose
SIg – Secretory Immunoglobulins
IgG – Immunoglobulin Gamma
IgA – Immunoglobulin Alpha
IgM – Immunoglobulin Meta
WHO – World Health Organisation
AAP – American Academy of Pediatrics
MicroRNA – Micro Ribonucleic Acid
ESPGHAN – European Society of Gastroenterology, Hematology and Pediatric Nutrition