UNDERSTANDING THE COMPOSITION AND PRODUCTION OF INFANT FORMULAS

Abstract

Infant formulas are specially designed food products to fulfill the nutritional needs of babies. While breast milk is the most beneficial option for babies, there are instances where it may be inadequate or not feasible. The purpose of infant formulas is to promote babies' growth and development by providing a nutrient profile akin to breast milk. Taking into account the compositional disparities between breast milk and cow's milk, the manufacturing of infant formulas is undertaken with precision, offering appropriate choices for different age groups. For newborns, formulas are available in liquid or powder form, whereas older babies may benefit from fortified solid food formulas. Additionally, hypoallergenic infant formulas have been developed for babies with cow's milk protein allergies. The production of infant formula adheres to strict quality and safety standards. The nutritional elements in infant formulas are formulated in accurate proportions to ensure they are well-balanced and nutritious. With the use of approved additives and rigorous oversight by health authorities, the quality and reliability of infant formulas are upheld. Infant formulas play a crucial role in babies' growth and development, enabling parents to provide suitable and healthy nutritional options for their babies. Consequently, this article delves into the significance of breast milk, a comparison of breast milk and cow's milk compositions, formula manufacturing technology, factors to consider in formula formulations, types of formulas available, and the legal regulations governing infant formulas.

Keywords: Breast milk, feeding; infant formula, compositions,

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I. INTRODUCTION

Nutrition is a basic need in every process from the beginning of life in the womb to the end of life. It is undisputed today that the baby is fed only with breast milk for the first six months, continued breastfeeding with additional nutrients after the sixth month, and continued breastfeeding until the end of the age of two [1]. Although the World Health Organization (WHO) recommends that a baby be exclusively breastfed for the first 6 months of life, only 44% of babies worldwide are breastfed. This shows that 56% take substitutes for breast milk [2]. Whether the child needs additional nutrition or not is determined by the growth curves, it is important to monitor the growth status of the baby and to decide on the time to give additional nutrition with the advice of a doctor [3,4].

Although breast milk is an ideal food for the baby, there may be situations such as the mother's inability to breastfeed the baby or not choosing to breastfeed, or the mother's working conditions not suitable for breastfeeding or insufficient breast milk. In these and similar situations, formulas with nutritional value and components that meet the nutritional needs of the baby in the first months of life are used as an alternative to breast milk. Complementary nutrition; It refers to the continuation of breastfeeding after the 6th month, as well as the introduction of other nutrients. Foods given in addition to breast milk are called complementary foods or supplementary foods. The period in which these foods are consumed is called the complementary feeding period. In order to protect and maintain the health of the baby or young child during this period, complementary foods should be adjusted according to the needs of the baby, and should be of high quality and reliable. While these foods can sometimes only be prepared from foods purchased specifically for the baby, sometimes they can be prepared appropriately for the baby from the foods consumed by the family [5]. Infant formulas are intended to partially or completely replace breast milk, so the nutritional value and components must be in composition to meet the nutritional needs of the baby in the first months of life. Given its physiological functions, including promoting cognitive development, enhancing innate and adaptive immunity, modulating immune response, preventing diabetes, maintaining gut health, and anti-inflammatory properties, breast milk is considered the gold standard for infant growth [6, 7].

II. HISTORICAL DEVELOPMENT OF INFANT FORMULA PRODUCTION

The oldest method used in infant feeding in cases where breast milk is insufficient is wet-nursing. B.C. to the records about his wet-nursing. It can be found until 2000 and this practice continued widely until the twentieth century. Over the centuries, the aristocracy of many cultures have adopted wet nurses to feed their babies [8]. In the nineteenth century, infant formulas began to be investigated as an alternative to infant feeding [9]. The development of the infant formula industry has been closely related to the increase in scientific studies [10]. German scientist Johann Simon conducted the first comprehensive milk analyzes comparing the components of human and cow milk in 1838 [11]. Following this, in 1865, a beverage was developed by the chemist Justus von Liebig by adding wheat, malt flour and potassium bicarbonate to cow's milk [8]. Liebig's formula led to the development of the infant formula market, and by 1883 there were 27 patented infant formulas, usually powders containing sugar, starch, and dextrin, designed to be added to milk [12]. However, since the importance of protein, vitamins and minerals needed by the baby is not known, these products were not included in the formula [13].

The first infant formula production was started in 1860 by Henri Nestle in Switzerland due to the increasing mortality rates as a result of the inability of working mothers to take care of their infants' nutrition. This food consisted of a mixture of cow's milk and grains and was called "Farine Lactee". The increase in birth rates after World War II led to the development of the infant formula industry. When birth rates decreased in the 1960s, manufacturers started to make campaigns to increase food consumption in developing countries. In the 20th century, infant formulas have become popular and there has been an increase in their use as an alternative to breast milk [14].

III. CHEMICAL COMPOSITION OF BREAST MILK AND COMPARISON WITH COW'S MILK

Breast milk comprises water, protein, fat, and mineral substances (Figure 1). Approximately 87% of breast milk is water, making it a crucial component. Fat constitutes about 3.8% of breast milk, contributing to approximately 50% of the total energy provided. The protein content of breast milk is 1.0%, with approximately 70% being whey proteins and 30% being casein. Lactose is the primary carbohydrate in breast milk, with the highest lactose content found among mammals, approximately 7%. Lactose supplies around 40% of the total energy in breast milk. Additionally, breast milk has a relatively low ash content, around 0.2% [15].

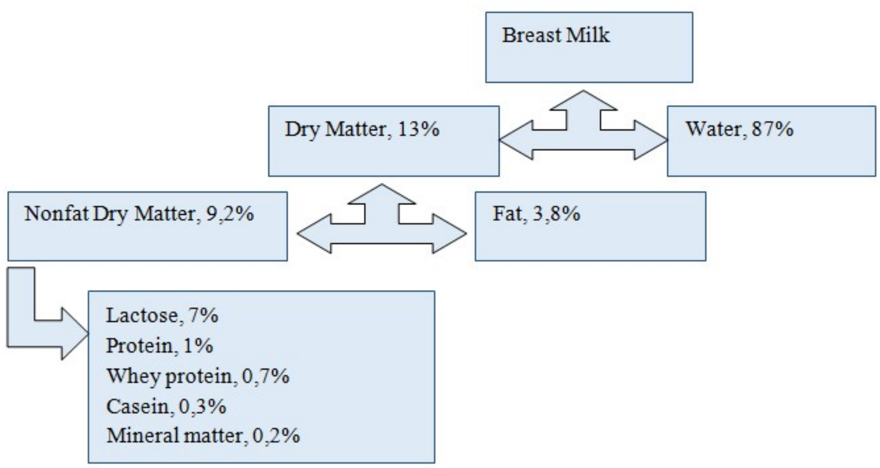

Figure 1: Composition of breast milk

The concentrations of dry matter components in breast milk can vary based on factors like genetics, physiology, nutrition, and environment. This variability is also observed in milk obtained from different animal species. As a result, different types of milk contain varying amounts of lipids, proteins, carbohydrates, minerals, vitamins, and enzymes [16].

Breast milk exhibits a unique chemical composition and biochemistry compared to bovine milk. It is characterized by higher levels of lactose and lower protein and ash content.

Unlike bovine milk, breast milk does not contain β-lactoglobulin and αs1-casein [9]. Table 1 presents the composition values of human and bovine's milk for comparison.

Table 1: Comparison of breast milk and bovine's milk

	Breast Milk	Bovine's milk
Gross composition (%)		
Protein	1.00	3.40
Caseins	0.3	2.6
Whey protein	0.7	0.8
Casein/Whey protein	30:70	80:20
Fat	3.80	3.50
Lactose	7.00	5.00
Total solids	12.40	12.50
Ash	0.20	0.70
Caseins (% of total)		
α S1-Casein	–	40
α S2 -Casein	–	8
β -Casein	85	38
κ -Casein	15	12
Micelle size (nm)	50	150
Whey protein (% of total)		
α -Lactalbumin	26	17
β -Lactoglobulin	–	43
Lactoferrin	26	trace
serum albumin	10	5
Lysozyme	10	trace
Immunoglobulins	16 (IgA)	10 (IgG)
Fatty acids (% of total)		
Saturated fatty acids	48.2	65.6
C_4 (Butyric)	–	3.5
C_6 (Caproic)	–	1.9
C_8 (Caprylic)	–	1.3
C_{10} (Capric)	1.4	2.5
C_{12} (Lauric)	6.2	2.8
C_{14} (Myristic)	7.8	10.7
C_{16} (Palmitic)	22.1	27.8
C_{18} (Stearic)	6.7	12.6
Monounsaturated	39.8	30.3
$C_{16:1}$ (Palmitoleic)	3.1	2.5
$C_{18:1}$ (Oleic)	35.5	26.5
$C_{20:1}$ (Gadoleic)	0.96	trace
$C_{22:1}$ (Cetoleic)	trace	trace
Polyunsaturated	10.82	4.5
$C_{18:2}$ (Linoleic)	8.9	2.9
$C_{18:3}$ (Linolenic)	1.2	1.6

$C_{18:4}$ (Parinaric)	–	trace
$C_{20:4}$ (Arachidonic)	0.72	trace
$C_{20:5}$ (Eicosapentaenoic)	trace	trace

Breast milk typically consists of various components, including approximately 26% α-lactalbumin, 26% lactoferrin, 16% immunoglobulins, 10% lysozyme, 10% serum albumin, and 12% other proteins [17]. The presence of casein and serum proteins in breast milk is particularly significant due to their immunomodulatory functions. In the later stages of lactation, the breast milk's casein-serum protein ratio is approximately 40:60 [18].

The lipids found in breast milk encompass triglycerides, phospholipids, sterols, and other minor components. Lipids play a crucial role as an energy source and provide nutrients essential for the development of the central nervous system [16]. Breast milk is abundant in essential fatty acids such as palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$, cis-9), linoleic acid ($C_{18:2}$, n-6), and α-linolenic acid ($C_{18:3}$, n-3). Among these, linoleic acid and α-linolenic acid are vital for the nervous system and retinal photoreceptors as they serve as precursors for arachidonic acid and docosahexaenoic acid [19].

Breast milk contains lactose as one of its main components, and its concentration is higher in breast milk compared to cow's milk. Moreover, breast milk contains carbohydrate-based bioactive oligosaccharides that enhance the absorption of calcium and zinc [20].

All water-soluble and fat-soluble vitamins are present in breast milk. In comparison to cow's milk, breast milk contains higher levels of vitamins A, E, C, nicotinic acid, and inositol. However, it has lower content of vitamins B_1, B_2, B_6, B_{12}, K, biotin, pantothenic acid, and choline (Table 2). Despite these variations, breast milk contains sufficient vitamins to support normal infant growth [9].

Minerals exist in the body in various chemical forms, including minerals, inorganic ions, salts, and as components of other organic molecules such as proteins, fats, and nucleic acids. They play essential roles in providing structural components to body tissues and forming important parts of many enzymes, contributing to various physiological functions. Breast milk and cow's milk have different mineral content, as shown in Table 2. Breast milk contains macrominerals like sodium, potassium, chloride, calcium, magnesium, phosphorus, and sulfate. Additionally, more than ten mineral substances, including iron, copper, and zinc, have been identified in breast milk. The primary minerals found in breast milk are phosphorus, potassium, and calcium (Table 2). It is noteworthy that breast milk generally has lower values of sodium, potassium, calcium, magnesium, and zinc compared to cow's milk [21].

Table 2: Vitamin and mineral content of breast and bovine's milk

	breast milk	bovine milk
vitamins		
Vitamin A (mg/mL)	0.53	0.37
Carotene (mg/mL)	0.24	0.21
Cholecalciferol (D) (mg/mL)	0.001	0.0008

Tocopherol (E) (mg/mL)	5.4	1.1
Vitamin K (mg/mL)	0.015	0.03
Thiamin (B_1) (mg/mL)	0.15	0.42
Riboflavin (B_2) (mg/mL)	0.37	1.72
Pyridoxine (B_6) (mg/mL)	0.10	0.48
Cobalamin (B_{12}) (mg/mL)	0.0003	0.0045
Niacin (mg/mL)	1.7	0.92
Folic acid (mg/mL)	0.043	0.053
Ascorbic acid (C) (mg/mL)	47	18
Biotin (mg/mL)	0.007	0.036
Pantothenic acid (mg/mL)	2.1	3.6
Inositol (mg/mL)	300	160
minerals		
Sodium (mg)	15	42.0
Calcium (mg)	33	112.0
Copper (µg)	39	20
Iodine (µg)	7	—
Iron (mg)	70	0.1
Magnesium (mg)	3	11.0
Manganese (µg)	0.003	6.0
Phosphorus (mg)	95	91.0
Potassium (mg)	55	145.0
Selenium (µg)	0.016	1.8
Zinc (mg)	0.16	0.4
Fluoride (mg)	0.01	—

IV. INFANT FORMULA TYPES

İnfant formula formulations have been developed as a result of technological developments. In terms of commercial forms, infant formulas are available in the market in ready-to-use, liquid concentrate and powder form. Baby formulas are produced in different varieties according to their intended use. Cow or goat milk-based, soy-based intensively hydrolyzed formulas are produced, formulas for premature and low birth weight infants and breast milk fortifiers are also available for special medical purposes [22, 23]. Follow-up formulas and supplementary foods are produced for this period when the content of breast milk or infant formula cannot meet the needs of the baby alone. Follow-up formulas take over the main liquid food function if breast milk is not taken during this period [22]. While preparing the infant formulation, cow's milk is generally used in the production of follow-on milk and infant formulas, and the difference between the composition of breast milk and cow's milk, the role of each of these components and the needs of the infant in terms of nutrition and immunity are taken as a basis [23].

1. **Formula Development in Infant Formula Production**

   Infant formulas are available in the market in three different forms [24]:

   - Powder: The smallest form of infant formula that requires mixing with water before feeding.
   - Liquid concentrate: Needs to be mixed with an equal amount of water.
   - Ready-to-eat: Baby formula that requires no mixing.

   For proper growth and maintaining good health, infant formulas should contain appropriate proportions of water, carbohydrates, proteins, fats, vitamins, and minerals. There are three main types of infant formulas [25]:

   - Milk-based formulas: Prepared from cow's milk, these formulas contain vegetable oils, vitamins, minerals, and iron, making them suitable for healthy full-term babies.
   - Soy-based formulas: Made with soy protein, vegetable oils (for fat calories), and corn syrup and/or sucrose (for carbohydrates). These formulas are suitable for infants with lactose intolerance or cow's milk protein allergy. However, they are not recommended for low birth weight or preterm infants or infants with colic.
   - Special formulas: Specifically designed formulas are required for low birth weight, premature infants, and infants with metabolic diseases or intestinal malformations. Examples include low-sodium formulas for infants intolerant to all proteins in their cow's milk-based formula, or pre-digested protein formulas for infants with allergies.

2. **Formulated Infant Formulas:** Formulated infant formulas are defined as products that meet the special nutritional needs of the baby in the first months of life, until the baby is introduced to appropriate complementary feeding, in cases where breast milk is not sufficient. It is also referred to as infant formula and is produced from cow's milk, whose composition has been made similar to breast milk. To change the composition of cow's milk, the total amounts of protein, fat, carbohydrate and mineral substances are adapted to the amounts in breast milk. In addition, certain vitamins and trace elements are added to the composition. Apart from this, the compositions of lipid and protein fractions can be changed, for example, milk fat can be mixed with vegetable oils to make the composition of fatty acids similar to that in breast milk, or vegetable oil can be used instead of milk fat [26].

3. **Follow-up Formulas:** Follow-up formulas are defined as products of 4th-6th days after the baby's birth. They are formulas designed to form part of a mixed diet regimen with the development of the digestive and excretory system. Continuation formulas; It constitutes the main fluid intake of infants in their increasingly varied diets from the sixth month, unless a different month is recommended based on the growth and development needs of the baby by an impartial healthcare professional specializing only in maternal and child nutrition, for special nutritional purposes. The protein content of follow-on formulas is slightly higher than that of formula formulas, due to the increasing need for protein as the baby grows and develops. Although it contains mostly milk fat in its composition, its vegetable oil rate is low [23].

4. **Foods Formulated for Premature Babies and Babies with Congenital Medical Conditions:** This type of food is considered in the category of diet foods for special medical purposes. Babies with premature, low birth weight, nutritional deficiencies and related health problems, and babies with congenital heart failure, fat absorption problems and metabolic disorders are included in this group. The energy value they provide is slightly higher than the formula formulas. The ratio of serum protein to casein is adapted to be 60:40 or 70:30. It contains serum protein, more cysteine, less methionine. This is quite convenient because the premature baby does not have the enzyme to convert methionine to cysteine. In premature babies fed with serum predominant protein, more growth increase was detected compared to those fed casein predominantly. The carbohydrate fraction consists of a mixture of maltodextrin and lactose. The usage rate of maltodextrin varies between 25-60%. Fat consists of a mixture of medium-chain triglycerides, which can be rapidly absorbed by the body, with vegetable oils and sometimes milk fat. The ratio of triglycerides in this mixture varies by 25-60% [23, 27].

V. CHANGES THAT MUST BE MADE IN COW MILK COMPOSITION IN PRODUCTION OF INFANT FORMULA

When designing formula infant formula, some changes must be made in the composition of cow's milk. Among the mineral substances, especially sodium and phosphorus content should be reduced, and the Ca:P ratio should be increased from 1.2 to 2. The protein content should be reduced and the casein: serum protein (20:80) ratio should be increased in favor of serum protein. The amount of carbohydrates should be increased, vegetable oils should be used instead of milk fat, certain vitamins and trace elements should be added. Adjusting the mineral content of cow's milk to be used in the production of formula infant formula is the most complex stage of formula production. For this, by adding carbohydrates to milk, the amount of other components that make up the total dry matter can be reduced. However, this type of application creates a 40-50% increase in the carbohydrate content of the food. Today, whey is used for this purpose [23]. However, since there is 8-10% mineral matter in whey dry matter, demineralization process is required. The use of demineralized whey also contributes to milk in terms of serum protein and lactose.

While the lactose in whey decreases the protein content of milk, an increase is provided in the casein: serum protein ratio in favor of serum protein, which is 60:40. In this way, the cystine level, one of the essential amino acids, also increases, and the contents of tyrosine and phenylalanine are reduced. The lactalbumin and lactoglobulin found in whey have a positive effect on normal growth and development as they contain important amino acids needed in the nutrition of babies. β-lactoglobulin is the protein with the highest rate (58%) among whey proteins and plays a role in the transmission of passive immunity in newborns and the regulation of phosphorus metabolism in the mammary gland. However β, -lactoglobulin is one of the substances that limits the use of cow's milk in the preparation of infant formula formulations and causes allergies in infants. In highly sensitive infants, this protein can cause allergic reactions even in very low amounts. For this reason, hydrolyzed casein and hydrolyzed whey formulas are recommended for infants. Lactalbumin , which is the second most abundant protein in whey proteins, αacts as a coenzyme in the biosynthesis of lactose, which is an important energy source for newborns. Pure - lactalbumin obtained from whey is used in infant formulas because it is similar to the basic protein in breast milk in terms of structure and composition [23].

Lactose, sucrose, dried corn syrups, glucose, maltodextrin, and wheat and corn starches are used to increase the total carbohydrate content of cow's milk. Lactose; It is preferred more because it prevents the development of pathogens, decreases the pH value of the stool, helps weight control due to its low sweetening power, and reduces the risk of dental caries. Maltodextrin, on the other hand, has some advantages because it reduces the osmolality of the product and is easier to digest than lactose [23]. By adding vegetable oils such as corn oil, soybean oil, coconut oil, safflower oil, hazelnut oil and mixtures of milk fat with vegetable oils to skimmed milk, the fatty acid composition of cow's milk is tried to be made similar to that of breast milk [26]. Since some of the vitamins, especially fat-soluble vitamins, are higher in breast milk, cow's milk should be enriched in this regard [23].
According to Montagne et al. [28], a typical infant formula should contain the following raw materials:

1. **Milk:** Skimmed or full-fat, in the form of liquid or powdered milk, cow's or buffalo's milk can be used.
2. **Casein:** Acid casein or neutralized potassium/calcium caseinate can be utilized.
3. **Whey Protein:** Concentrated (WPC), isolate (WPI), partially demineralized serum protein, or partially hydrolyzed serum protein can be included.
4. Isolated soy protein, carob seed protein, and amino acids (elemental formula) may also be added to the infant formula.
5. **Lactose:** It can be added in the form of maltodextrin, corn syrup, sucrose (powder or syrup).
6. **Vegetable Oils:** Non-hydrogenated vegetable oils can be used.
7. **Emulsifiers/Stabilizers:** Lecithin (soy), monoglycerides, and diglycerides may be included in the formula composition.
8. **Mineral Salts:** Potassium, sodium, calcium, and magnesium such as carbonates, citrates, phosphates, or chlorides.
9. **Micronutrients:** Vitamins, amino acids, KI, FeSO4, ZnSO4, CuSO4.

These raw materials play a crucial role in formulating infant formulas that meet the nutritional needs of babies. Moreover, the following optional ingredients can be added to infant formulas:

10. GOS, FOS, and inulin can be included to provide probiotic properties [29, 30, 31].
11. Probiotic microorganisms such as *Bifidobacterium animalis sp. lactis, Lactobacillus rhamnosus GG, Bifidobacterium longum BL999*, and/or other strains of *L. rhamnosus* may be added [30, 32, 33, 34].
12. Specialty oils like deodorized high docosahexaenoic acid (DHA) fish oil, LCPUFA oils, medium-chain triglycerides, interesterified palm oil, and sphingolipids can be used [35, 36].
13. Locust bean gum and corn or potato starch may also be added [37, 38].

For newborn babies, α-lactalbumin (α-La) holds significant nutritional value as an excellent source of essential amino acids like tryptophan and cysteine. Therefore, it is commonly used to supplement infant formulas [39]. The addition of whey protein fractions enriched with α-La aims to mimic the composition of breast milk [40]. Recent research has revealed that α-La influences gut microbiota, mineral absorption, and plays a physiological role in the immune system [41]. As a result, whey protein fractions enriched with α-La and reduced β-lactoglobulin content are increasingly utilized in the production of infant formulas [40].

The usage of sesame and cottonseed oil in infant formulas is prohibited due to their potential adverse effects. Sesame oil contains sesamolin and sesamin, which have been identified as substances causing contact dermatitis [42]. Cottonseed oil may contain cyclopentenic fatty acids, which could negatively impact fatty acid metabolism, leading to the desaturation of fatty acids [43]. The total fat content in infant formulas should not exceed 20 g per 100 g of the formula, specifically with regards to lauric and myristic acid. The standards also state that the trans fatty acid content should not exceed 3 g per 100 g of the total fat content, and the erucic acid content should not exceed 1 g per 100 g of the total fat content [44, 45]. Palm oil (palm olein) and soybean oil, which contain palmitic and oleic acids similar to those in breast milk, are often combined with other oils in infant formulas.

The primary carbohydrates in breast milk consist of galacto-oligosaccharides, lactosucrose, lactulose, and lactitol. After the first week of breastfeeding, bifidobacteria are reported as the predominant gastrointestinal bacteria, while Bacteroides spp. are observed to develop a more diverse microbiota [46]. Oligosaccharides, which are the third-largest component of breast milk, are virtually absent from cow's milk and most infant formulas. However, a combination of neutral short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) in infant formulas has been shown to reduce the incidence of atopic dermatitis and infectious episodes during the first 6 months of life [47].

In general, the mean daily intakes of calcium, magnesium, sodium, potassium, and iron for formula-fed infants meet the recommended values, except for iron intake when using iron-fortified formulas [48]. In the absence of breast milk, iron-fortified infant formulas are the most suitable alternatives for feeding healthy, full-term infants in their early years of life [49].

VI. IMPORTANCE OF BREAST MILK AND COMPARISON WITH INFANT FORMULA

Breast milk is a highly nutritious food that fulfills all the essential needs of newborns and growing babies. Compared to formula-fed infants, breastfed babies tend to have lower rates of obesity, diabetes, and cardiovascular diseases. Breast milk contains various proteins, such as lactoferrin, α-lactalbumin, milk fat globule membrane proteins, and osteopontin, which exhibit different bioactivities, ranging from protecting against infections to aiding nutrient absorption from breast milk. To mimic these benefits, infant formulas include protein fractions enriched with these bioactive proteins [50, 51].

As scientific research on infant formulas and technological advancements progress, formulas have come closer to replicating the composition of breast milk. Consequently, formula-fed infants' overall development has become more comparable to breastfed infants. These improvements involve modifications in whey, the addition of nutrients like taurine, nucleotides, docosahexaenoic acid (DHA), and arachidonic acid; as well as the inclusion of prebiotics (fructo-oligosaccharides/galacto-oligosaccharides) and lutein. However, despite these advancements, there still seem to be differences in both short-term (e.g., illness, cognitive development) and long-term (e.g., obesity, diabetes, cardiovascular disease) outcomes between breastfed and formula-fed infants. While various components of breast milk have exhibited bioactivity in vitro, there is limited evidence from clinical studies to directly incorporate them into infant formulas [50].

Breast milk exhibits a bacteriostatic effect against Escherichia coli bacteria, attributed to the iron-binding protein lactoferrin, which is abundant in breast milk [52]. Lactoferrin acts as an iron-binding agent, depriving iron-dependent pathogenic bacteria of this essential nutrient, inhibiting their growth. Additionally, lactoferrin acts as a bactericide, capable of killing pathogens like Vibrio cholera and Streptococcus mutans [53]. Working synergistically with lysozyme, another antimicrobial component in breast milk, lactoferrin even inhibits resistant Gram-negative bacteria [54]. Clinical studies involving infant formulas enriched with bovine lactoferrin have shown reductions in upper respiratory disease at 6-12 months of age [55]. Furthermore, research [56] demonstrated a significant decrease in sepsis cases in premature infants after receiving oral supplements of bovine lactoferrin.

Similarly, lysozyme, present in both preterm and term infants' feces, exhibits antimicrobial activity within the gut of breastfed infants [57].

Breast milk casein micelles primarily consist of β-casein and k-casein, with smaller amounts of αS-casein. Notably, k-casein and its proteolytic fragment, glycomacropeptide (GMP), possess antimicrobial effects. They have the ability to bind to pathogens, impeding infection [58].

The milk fat triglycerides are encased in protein-containing membranes, several of which have known antibacterial and antiviral activities [59].

VII. CHANGES DURING THE PRODUCTION AND STORAGE OF INFANT FORMULAS

Infant formulas are carefully formulated, considering potential changes or losses that might occur in the raw materials during production and storage, with a safety margin. Protein and lactose contents are typically kept slightly higher than those found in breast milk [23].

Foods, including formulas, are susceptible to the Maillard reaction due to their high sugar and lysine-rich protein content. The Maillard reaction is catalyzed by high temperatures and prolonged storage, resulting in browning reactions [60]. During heat treatment, the ε-amino group of lysine, bound to proteins, reacts with lactose through the Maillard reaction, especially under severe heating conditions. This results in the formation of stable "lactulosyl-lysine," which is less biologically available to the body. As infant formulas contain relatively high levels of lactose, they may experience a higher loss of lysine compared to normal milk. Spray drying, a common method for formula production, can cause up to a 10% loss of lysine. For formulas prepared with glucose for babies with lactose intolerance, which is more reactive than lactose, lysine loss during traditional spray drying can reach up to 70%. However, controlled heat application can reduce this loss level to around 15%. In foods containing enzymatically degraded proteins, heat treatment can lead to the formation of glycosylated peptides. Maillard reactions may also continue during storage [23, 60].

During the sterilization process of liquid infant formulas, lysinolalanine is formed when lysine reacts with serine phosphate. Among different sterilization methods, bottle sterilized formulas show the highest amount of lysinolalanine, while UHT sterilized formulas exhibit the lowest levels. This formation of lysinolalanine is considered a toxicological concern rather than a nutrient loss issue [23].

In heat-treated foods with high lactose content, such as infant formulas, the formation of lactulose from lactose occurs at a higher rate during bottle sterilization compared to UHT sterilization. Prolonged storage at high temperatures can lead to a slight increase in lactulose content in sterilized infant formulas. While lactulose supports the development of Bifidobacteria in infants' intestines, excessive amounts (200 mg/100 ml) can cause lactose-like intolerance. Additionally, the lactulose used by Bifidobacteria is metabolized into organic acids, like lactic acid and acetic acid, reducing intestinal pH and increasing volatile fatty acid production. This can result in increased osmotic pressure and a laxative effect [23, 61].

During the sterilization process of liquid infant formulas, there may be losses of vitamin C, thiamine, folic acid, and vitamin B6. Bottle sterilization tends to cause higher rates of vitamin loss compared to UHT sterilization. To account for potential vitamin losses, formulas are formulated to contain higher levels of vitamins than what is indicated on their labels. A tragic example of this issue occurred in Israel when a German-made infant formula led to vitamin deficiencies in 15 babies, resulting in fatalities. In response, company officials stated that they were unaware of the potential vitamin losses caused by heat during the sterilization process [23, 62].

VIII. INFANT FORMULA PRODUCTION TECHNOLOGY

Infant formulas are composed of various ingredients, including milk, milk components, soy protein isolate, carbohydrates, fats, vitamins, minerals, and other food additives. The production process involves several steps, starting with the acceptance of unprocessed raw materials like raw milk or liquid whey. Standard dairy processing methods, such as pasteurization, homogenization, and standardization, are commonly applied [63].

The majority of infant formulas are produced in powder form, which requires dissolving the powder in water before feeding the baby. There are two main production methods: wet processing and dry processing. Wet processing ensures a homogeneous mixture, while dry processing is more cost-effective with lower investment expenses. Often, a combination of these two methods is employed. Water-soluble substances are added to the milk before drying, while less soluble components are added in powder form after drying, achieving the desired composition of the formula [14, 62].

1. **Wet Process:** Once the raw material components are added, the dry mixture is combined with water to create a liquid mixture. The subsequent processes applied depend on the specific product composition and may include pasteurization in a plate heat exchanger (at temperatures of 71-74°C for 15-25 seconds) or sterilization by direct steam injection (at temperatures of 105-125°C for at least 5 seconds). The type of heat treatment used varies according to the product's composition. For instance, foods containing starch are pasteurized at 74.4°C for 25 seconds. After the heat treatment, the liquid formula is thickened through an evaporator and then either dried or stored aseptically. For technological reasons, the concentrate is preheated at temperatures up to 70°C before

drying. This preheating process also serves to further reduce the number of vegetative cells in the formula [14, 63].

2. **Dry Process:** The purpose of drying the liquid concentrate is to achieve a low water activity (<0.3) and obtain a powder with an extended shelf life. Among the available technologies, spray drying is the most commonly used method. However, the roller mill method is not recommended due to its negative impact on microbial quality and the irreversible changes it causes in food components [14, 23].

   In the production of milk-based powdered infant formula, the following steps are typically followed: After undergoing separation, clarification, and deaeration processes, the milk is pasteurized at 75°C for 20 seconds. Skim milk is then mixed with vegetable oils, demineralized whey, fat-soluble vitamins, emulsifiers, and stabilizing agents. Water-soluble vitamins and mineral substances are generally added before the drying process, although they can also be added after drying in powder form. The blended mixture undergoes homogenization at a pressure of 150-200 kg/cm2. Heat treatment is then applied to the mixture at 110°C for 60 seconds, and the lactose-rich content results in a maximum of 45% dry matter before it is dried through the spray drying method. For the production of instant powder, fluid bed dryers are utilized. Liquid foods require sterilization before aseptic packaging. This is accomplished through either the UHT method (150°C for 3 seconds) or the classical method (115°C for 10-15 minutes) [23].

IX. USE OF PROBIOTICS, PREBIOTICS AND SYNBIOTICS IN INFANT FOODS

Although the intestines of the newborn baby have a unique flora, it changes rapidly with the nutrients taken immediately after birth. The first microbial colonization stage is very important in the development of the immune system. At this stage, the gastrointestinal system takes shape and the immune system begins to perform its normal functions. The normal intestinal flora of humans consists of $^{10-12}$ microorganisms/g and the content contains more than 400 bacterial species [64]. The main source of the bacteria that make up the intestinal biota of the baby are the microorganisms found in the mother's birth canal and the people in contact with the baby's close environment. There are many external and internal factors such as whether the mother consumes foods containing probiotic bacteria, type of delivery, gestational age and the baby's initial feeding style (breast milk or formula), health of the newborn baby, immune system, gastrointestinal system transit time, pH and stress. factors affect bacterial growth [65, 66]. Low birth weight premature babies born by cesarean section are delayed in starting breast milk during their stay in the intensive care unit, and contamination with pathogenic microorganisms may occur during this period [67].

Bifidobacteria is predominate in the intestinal biota of breastfed infants, while *Enterobacter species* are dominate in the intestines of formula-fed infants. Although *Bifidobacteria are present* in the biota of infants fed with formula for six months, it is less than that of breastfed infants. Intestinal biota of one-year-old children fed with breast milk and formula is similar to each other and is close to adult biota [66]. Bifidobacter group probiotics have been added to infant formulas for 15 years and no side effects have been observed in this period. Bifidobacteria are thought to play a beneficial and important role in maintaining the proper balance of the normal intestinal flora [68]. *Lactobacilli* are the most widely used probiotics today. *Lactobacilli* can be found as probiotics in infant formulas, probiotic-added milk and various pharmaceutical preparations [69, 70].

The intestines are the largest immune organ in the body, and intestinal bacteria are in constant interaction with immune system cells. Intestinal lymphoid tissue of experimental animals raised in a bacteria-free environment cannot develop and their circulating immunoglobulin concentrations are low [66]. Probiotics not only strengthen the immune system in the gastrointestinal tract, but also have an effect on the systemic immune response. The best example of this is the strengthening of the immune response to vaccines. Positive results have been obtained from the use of probiotic bacteria such as *Lactobacillus* GG together with typhoid, oral polio and rotavirus vaccines [66].

Many children die due to diarrhea in the world. Rotavirus is the most common cause of acute diarrhea in developed societies. *L. rhamnosus GG (lgg)*, *Lactobacillus reuteri*, *Lactobacillus casei* and *Bifidobacterium animalis spp lactis* are counted among the strains with proven efficacy in these diarrheas. Probiotic bacteria can prevent acute diarrhea by preventing the attachment of pathogens, strengthening immunity, regulating motility, suppressing secretory mechanisms and increasing mucin production. It has been shown that when *Bifidobacterium bifidum* and *Streptococcus thermophilus* are added to infant formulas, the frequency of diarrhea decreases by 24% [70].

Diarrhea encountered with antibiotic use in infants is caused by *Clostridium difficile.* In the treatment of the disease, *Lactobacillus acidophilus, L. plantarum, L. casei* and *Saccharomyces boulardii* are used. It is effective in the treatment by binding the toxins of *C. difficile* [72, 73]. Neonatal necrotizing enterocolitis (NEC), a serious gastrointestinal disease of unknown cause, of the neonatal period; It is characterized by abdominal distention, bilious vomiting, bloody stool, lethargy, apnea and bradycardia [71, 74]. *Clostridium perfringens* was isolated in 40% of babies who developed NEC, and the rate of lactobacilli was found to be decreased. Lin *et al*. (2005) showed that the frequency of NEC decreased by 63% in babies fed with formula containing *Lactobacillus acidophilus* and *Bifidobacterim infantis* in addition to breast milk. It has been observed that the use of probiotic and prebiotic added formula in premature infants increases the gastric emptying rate similar to breast milk [71, 75]. Probiotics are also effective on weight gain, especially in premature babies. In a placebo-controlled study, when *Bifidobacterium lactis was given* to premature infants receiving antibiotic therapy, it was found that this group had 4 times better weight gain than the control group [76]. Infantile colic seen in the first 6 months of infancy; It is a disease in which symptoms such as restlessness, crying spells, blushing during crying, and pulling the legs of the child towards the abdomen are seen periodically in healthy and well-nourished children. In studies on this subject; In addition to breast milk, it has been reported that formula compositions containing casein hydrosylate, *Lactobacillus reuteri,* fructose and oligosaccharides reduce crying spells [77, 78, 79, 80].

In order to determine the role of probiotics in the prevention and treatment of atopic dermatitis, probiotics were used in a study conducted on 1898 children from the neonatal period to the age of 13. According to the results of the study, it was determined that probiotics reduced the development of atopic dermatitis by 61% in the first 2 years [80]. Lodinova et al. (2003) determined that when pregnant women and newborn babies were given *Lactobacillus rhamnosus GG,* there was a 50% reduction in atopic eczema compared to those given placebo [81]. It has been determined that probiotics reduce the incidence of constipation, acute diarrhea, and increase tolerance to the new diet in infants after weaning [82].

Prebiotics are non-digestible carbohydrates that provide beneficial effects to the host that selectively increase the growth and/or activity of bacterial species in the colon. Inulin, Lactulose, Fructo-oligosaccharides (FOS), Galacto-oligosaccharides (GOS), Resistant starch, Lactosucrose, Gluco-oligosaccharides, Raftiline, New (Neo) sugars, Oligomate, Xylo-oligosaccharides, Palatinose, Pyrodexittoolose, -oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides can be counted as prebiotic components [73, 83]. Prebiotics stimulate the growth of beneficial species (such as *Lactobacillus* , *Bifidobacterium)* in the normal intestinal microbiota more, and prevent the proliferation of potential pathogenic microorganisms such as toxin producing *Clostridiums*, proteolytic *Bacteroides* and toxigenic *Esherichia coli* [84]. It is a known issue that Bifidobacteria are intense in the intestinal flora of breastfed babies. About 50 years ago, breast milk oligosaccharides were identified as one of the important bifidogenic factors in breast milk. The oligosaccharide content of breast milk is approximately 0.7-1,2 g/l and is seen as an important factor in the defense of newborn babies against infections. Studies have shown that there is an increase in the amount of bifidobacteria and lactobacilli in the intestinal microflora thanks to the prebiotics added to infant formulas. Addition of GOS and FOS to infant formula shows a growth factor effect for bifidobacteria, such as breast milk oligosaccharides.

Prebiotic mixtures of specific galactooligosaccharides (GOS) and fructooligosaccharides (FOS), which are similar to the oligosaccharides in breast milk and stimulate the growth of bifidobacteria and lactobacilli, have been identified in infant formulas. Bifidobacteria of prebiotics These bacteria are used together with prebiotics to provide better results. Addition of fructo- and galactooligosaccharides to premature infant formulas stimulates the proliferation of bifidobacteria and provides soft and frequent defecation [66]. In addition, the ability of various probiotics and prebiotics to increase calcium absorption and improve the bone density levels of children has been demonstrated in clinical trials [85].

X. INFANT FORMULA STANDARDS AND LEGAL REGULATIONS

Infant formulas are subject to legal regulations accepted by international organizations like the United Nations Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Commission and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). Each country also has its own regulations, often based on these international standards.

In the United States, the Food and Drug Administration (FDA) adopted regulations for adapted infant formulas in 1985, following recommendations from the American Academy of Pediatrics. Similarly, in Europe, the European Union established standards for infant formulas made from cow's milk or soy through the Infant Formulae Directive (2006). This directive considers scientific evidence and sets guidelines for the addition of new ingredients to infant formulas.

The European Communities Regulations of 1998 were developed based on widely accepted scientific data regarding infant formulae and follow-on formulae. These regulations cover various aspects of food production, importation, and marketing, including regulations related to diseases, pesticides, veterinary drug residues, food production, and marketing hygiene, as well as the admittance of third-country food and hygiene certification for authorities.

Furthermore, the Commission Directive 2006/141/EC, known as the Recast Directive, provides clear guidelines on the composition, quality, and labeling requirements for infant formulas sold within the European Union. This directive ensures that infant formulas in the market meet specific standards for quality and safety.

The Turkish Food Codex (TFC) Regulation (Official Gazette: 29.12.2011-28157) serves as the fundamental legislation for baby and infant foods, as established in Article 23 of Law No. 5996. This regulation is further complemented by the TFC Regulation for Special Purpose Foods (Official Gazette: 22.04.2002-24734), which specifically addresses baby and infant foods.

Within the TFC framework, horizontal and vertical regulations are put in place to ensure the proper sampling, analysis methods, and safety standards for various food categories. Vertical food codex refers to legislation specifically designed for particular food groups, substances, or materials in contact with food. For baby and infant foods, specific arrangements are made through the TFC Infant Formulae Regulation (Official Gazette: 15.08.2014-29089), TFC Follow-On Formulae Regulation (Official Gazette: 15.08.2014-29089), and TFC Baby and Infant Supplementary Foods Regulation (Official Gazette: 01.11.2007-26687).

On the other hand, horizontal regulations encompass general provisions that are applicable to all types of foods and materials in contact with food. By implementing both horizontal and vertical regulations, Turkey ensures the safety, quality, and proper labeling of baby and infant foods in accordance with established standards.

XI. CONCLUSION

The importance of infant nutrition becomes even more significant during the period of rapid growth and development that continues from birth until the age of two. During the first six months, the baby is exclusively breastfed, but as the baby reaches 6-12 months of age, complementary feeding becomes crucial as it fulfills 50% of the nutritional needs that breast milk alone may not provide. There are various reasons why breast milk may not be available or sufficient for the baby, such as the mother's mental well-being, the presence of diseases like tuberculosis, HIV, cancer that could be transmitted to the baby through breast milk. In cases where breast milk is not available or insufficient, baby formulas serve as an alternative. In the production of formulas, it is vital to ensure that each nutrient is added in the appropriate amount that will benefit the baby. To ensure the safety of baby and infant foods, proper and hygienic manufacturing, preparation, processing, storage, and preservation are crucial.

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