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Effect of processing on polyamine content and bioactive peptides released after in vitro gastrointestinal digestion of infant formulas

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ABSTRACT

This study examined the influence of processing on polyamines and peptide release after the digestion of a commercial infant formula designed for children during the first months of life. Polyamine oxidase activity was not suppressed during the manufacturing process, which implicates that polyamine concentrations were reduced over time and during infant formula self-life. In gel electrophoresis, in vitro gastrointestinal digestion of samples with reduced amount of enzymes and time of digestion shows an increase in protein digestibility, reflected in the increase in nonprotein nitrogen after digestion and the disappearance of β-lactoglobulin and α-lactalbumin bands in gel electrophoresis. Depending on the sample, between 22 and 87 peptides were identified after gastrointestinal digestion. A peptide from β-casein f(98–105) with the sequence VKEAMAPK and antioxidant activity appeared in all of the samples. Other peptides with antioxidant, immunomodulatory, and antimicrobial activities were frequently found, which could have an effect on infant health. The present study confirms that the infant formula manufacturing process determines the polyamine content and peptidic profile after digestion of the infant formula. Because compositional dissimilarity between human milk and infant formula in polyamines and proteins could be responsible for some of the differences in health reported between breast-fed and formula-fed children, these changes must be taken into consideration because they may have a great effect on infant nutrition and development.

Key words: infant formula, polyamine, peptide, simulated gastrointestinal digestion, mass spectrometry

INTRODUCTION

Breast milk has a complex composition of nutrients and bioactive components designed to fulfill the needs of the growing infant. In recent years, the infant food industry has made an effort to develop infant formulas that are more similar to human milk to improve the nutrition of infants who are not breastfeeding.

Protective compounds, such as cytokines, oligosaccharides, and even microbes, in breast milk provide the newborn with the means to adapt to the environment (Gueimonde et al., 2007; Newburg and Walker, 2007). Among the bioactive compounds found in breast milk are polyamines, such as, spermidine, spermine, and putrescine, as well as bioactive peptides released during milk protein digestion. Polyamines have a positive effect on the development of the gastrointestinal tract (Larqué et al., 2007) and immune system (Pérez-Cano et al., 2010; Gómez-Gallego et al., 2014b). The levels and effects of these compounds in infant formulas compared with human milk are of special interest, because their concentrations are lower than in human milk (Buts et al., 1995). Moreover, dietary proteins are a source of biologically active peptides that are inactive within the sequence of parent protein and can be released during gastrointestinal digestion or food processing. Once bioactive peptides are liberated, they may act as regulatory compounds. Bioactive peptides are widely distributed among milk protein sequences (Clare and Swaisgood, 2000), which can be released during digestion in vivo. However, changes that take place in protein structure during the manufacturing of infant formulas can influence protein digestion and peptide liberation (Korhonen et al., 1998).

Technological processes used in food manufacturing affect the functional, nutritional, and biological properties of food components. Depending on the intensity of the heat treatment, the nutritive value of proteins can be affected in a positive or negative way (Korhonen et al., 1998) and, by extension, can affect other related compounds.

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The structure of milk is greatly altered depending on the various mechanical and thermal steps of the processing chain (Michalski and Januel, 2006). Heating and homogenization are the most common and most widely used methods capable of modifying proteins during infant formula manufacturing. Among the different physical and chemical changes, a great deal of attention has been focused on the covalent interaction of protein–carbohydrate via the Maillard reaction. During this reaction, the conjugation of a reducing carbohydrate to the 3-amino group of lysine occurs spontaneously under heating conditions (Corzo-Martínez et al., 2012). Some studies have shown contradicting effects regarding the Maillard reaction. On one hand, glycation can lead structural changes, which could generate new enzymatic cleavage sites (Corzo-Martínez et al., 2012; Joubran et al., 2015) modulating protein digestibility and peptides release after digestion. On the other hand, conjugation could limit enzymatic accessibility through steric hindrance (Joubran et al., 2015). These 2 counteracting effects could explain the different digestive patterns reported for other authors. Moreover, heat treatment changes the effect of homogenization on milk structure (Michalski and Januel, 2006). The main effect of homogenization on soluble milk components is the disruption of casein micelles in micellar form or as fragments. Homogenization seems to improve milk digestibility. However, heat treatment changes the effect of homogenization on milk structure, and it has been reported that infants better digest native human milk fat globules than homogenized droplets from infant formula (Michalski and Januel, 2006). As concluded by Michalski and Januel (2006), the structural consequences in milk proteins seem to depend on the sequence of the homogenization and heat treatments, but they are rather controversial because of the various treatments applied and to the different procedures used in the food industry.

The aims of this study were to evaluate how formula processing influences polyamine content and peptide release after digestion. Furthermore, the behavior of the polyamine oxidase activity of the milk and the digestibility of proteins were studied. The results of this work could be a preliminary step to improving infant formula composition, which could promote better health status of children fed with infant formulas during the first months of life.

MATERIALS AND METHODS

Samples

The infant formula samples used in this study were supplied by Hero España S.A. (Alcantarilla, Spain) at different representative stages along the manufacturing process. Figure 1 shows the flow diagram of the infant formula manufacturing process and the steps at which the samples were taken. The samples were (**F1**) cow milk used as raw material in infant formula processing; (**F2**) cow milk after skimming and the first thermal treatment; (**F3**) concentrated milk after the second thermal treatment; (**F4**) concentrated infant formula after the last thermal treatment; (**F5**) the infant formula final product; and (**SW**) milk whey used as an ingredient. Whey was demineralized sweet whey from cow milk, with a content of lactose around 70%, added as an ingredient to increase milk serum proteins in the final product to 60% of total proteins.

One kilogram of powdered infant formula (F5) and 500-mL aliquots of liquid samples (F1, F2, F3, F4, and SW) were taken from 5 different batches separately. Liquid samples were lyophilized, and all of the samples were preserved at −20°C until analysis.

Determination of Moisture and Nitrogen

Moisture (method 964.22) along the infant formula manufacturing process and nitrogen (N) content using the micro-Kjeldahl procedure (method 955.04) were determined in the samples and digestions using official AOAC methods (AOAC, 1990). Protein calculations were made using 6.25 as the conversion factor. Nonprotein nitrogen in the samples and digestions was estimated using the micro-Kjeldahl method after dissolving 20 g of the sample in 100 mL of 15% TCA for milk protein precipitation and filtration.

Analysis of Polyamines

An HPLC method using a diode array detector was used. The HPLC system consisted of a Waters 2690 system connected to a Waters 910 detector. The analytical column was a Spherisorb 5.0-μm ODS2, 4.6 mm × 150 mm (Waters Corp., Milford, MA). Detection was performed at 254 nm.

The samples were diluted 10 times with a solution of TCA as described by Nishibori et al. (2007), but the concentration was adjusted to 15% for milk protein precipitation. The samples were homogenized using gentle agitation for 30 min (Pollack et al., 1992). After centrifugation at 13,000 × g for 15 min at 4°C, the supernatants were filtered using 0.45-μm membrane filters (Whatman, Brentford, England) and dansylated by adapting the method described by Buts et al. (1995). Clear supernatant (1 mL) was basified by adding 250 μL of saturated sodium carbonate and 1 mL of