DEVELOPMENT OF GOAT MILK INFANT FORMULAS BY MACRONUTRIENT MODIFICATION AND THEIR QUALITY EVALUATION

by

CATHERINE OBIANUJU MADUKO

(Under the Direction of Young W. Park)

ABSTRACT

Compositional differences between goat and human milk necessitate simulation of goat milk to human milk including the macronutrient compositions such as protein and fat. Macronutrients of goat milk were modified by protein fractionation and lipid modification to make the formulated products homologous to human milk.

Goat milk proteins were fractionated in two steps by ultrafiltration process. The effects of heat or frozen treatment, as well as the first-step and second-step fractionations using different membrane sizes were studied.

Infant formula analogues were prepared by modifying fatty acid profiles and sterol contents of goat milk with different vegetable oil blends to simulate the fat moieties of human milk for infant feeding. Fatty acid profiles and sterol contents of the formula milks were analyzed. The formula analog composed of 2.5:1.1:0.8 blend of coconut, safflower, and soybean oils had the best similarity to fatty acid profile, total sterol, cholesterol and phytosterol content of human milk.
Human milk fat substitutes were produced from vegetable oil blends in a packed-bed bioreactor. The effects of substrate molar ratio, reaction temperature and incubation time on palmitic acid incorporation at the sn-2 position were determined by the response surface methodology. Human milk fat substitutes obtained at optimal conditions of 3 substrate molar ratio (mol vegetable oil/ mol tripalmitin), 14.4h incubation time, and 55°C reaction temperature had 40.8% palmitic acid incorporation at the sn-2 position.

Structured lipids (SLs) for infant milk formulation were produced by enzymatic interesterification of tripalmitin with vegetable oil blends and fish oil. The SLs were characterized by fatty acid content and structure, melting profiles, oxidative stability index, free fatty acid (FFA) concentration, and tocopherol content. The structured lipids after purification by distillation had melting profiles, oxidative stability index, and initial FFA concentration which were similar to those of the starting oils. The fatty acid composition and structure of the SLs were similar to those of human milk fat. Oxidative stability of the SLs was improved with tocopherol addition as antioxidants and was comparable to that of the vegetable oils and oil blends.

INDEX WORDS: Goat milk, Infant formula, Protein fractionation, Ultrafiltration, Human milk fat substitute, Fatty acid, Cholesterol, Enzymatic interesterification, Structured lipids, Response surface methodology, Oxidative stability, EPA, DHA.
DEVELOPMENT OF GOAT MILK INFANT FORMULAS BY MACRONUTRIENT MODIFICATION AND THEIR QUALITY EVALUATION

by

CATHERINE OBIANUJU MADUKO

B.S., University of Nigeria, Nigeria, 2002

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2007
DEVELOPMENT OF GOAT MILK INFANT FORMULAS BY MACRONUTRIENT MODIFICATION AND THEIR QUALITY EVALUATION

by

CATHERINE OBIANUJU MADUKO

Major Professor: Young W. Park
Committee: Casimir C. Akoh
Joseph F. Frank
Robert L. Shewfelt
Yao-Wen Huang

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2007
DEDICATION

To my spouse, David Carver Peck, with whose help and encouragement the completion of my program was successful.
ACKNOWLEDGEMENTS

All thanks to Almighty God, by whose grace I was able to start and complete this program. A special thanks to my family, both in Nigeria and in the United States for their help and prayers and for their encouragement in my quest for knowledge.

Great thanks and appreciation goes to my major advisor, Dr. Y.W. Park, for his academic assistance, professional advice, encouragement, honesty, kindness, and relentless patience with me throughout this program. Lots of thanks to the members of my committee: Dr. C. Akoh, Dr. J. Frank, Dr. Y. Huang, and Dr. R. Shewfelt, for their excellent academic guidance, prompt professional advice and laboratory assistance during this program. Many thanks also to Dr. R. Toledo, Dr. P. Koehler, Dr. Eitenmiller, Dr. W. Kerr, Dr. L. Wicker, Dr. M. Harrison, and Dr. R. Singh for their academic and laboratory advice.

Deep appreciation to David Peck, Brenda Jennings, Anne Morrison, and Ruth-Ann Morrow, for their technical guidance and advice, which made my laboratory work a great success. Sincere appreciation also to my laboratory colleagues for their time and assistance during my laboratory work.

Finally, warm appreciation to my spouse, David Carver Peck, for his time, laboratory guidance and assistance, academic advice and encouragement, professional mentoring, selfless road assistance, financial assistance, and profound patience and understanding with my long stay in the laboratory, throughout the period of this program. Thank you David, and God bless you.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>COMPARISON OF FATTY ACID AND STEROL COMPOSITION OF MODIFIED GOAT MILK PRODUCTS FOR INFANT FORMULAE</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>ENZYMATIC INTERESTERIFICATION OF TRIPALMITIN WITH VEGETABLE OIL BLENDS FOR FORMULATION OF CAPRINE MILK INFANT FORMULA ANALOGS</td>
<td>113</td>
</tr>
<tr>
<td>5</td>
<td>ENZYMATIC PRODUCTION OF INFANT MILK FAT ANALOGS CONTAINING PALMITIC ACID: OPTIMIZATION OF REACTIONS BY RESPONSE SURFACE METHODOLOGY</td>
<td>142</td>
</tr>
<tr>
<td>6</td>
<td>CHARACTERIZATION AND OXIDATIVE STABILITY OF A STRUCTURED LIPID: INFANT MILK FAT ANALOG</td>
<td>170</td>
</tr>
<tr>
<td>7</td>
<td>PRODUCTION OF INFANT FORMULA ANALOGS BY MEMBRANE FRACTIONATION OF GOAT MILK: EVALUATION OF TEMPERATURE TREATMENT ON MEMBRANE PERFORMANCE</td>
<td>201</td>
</tr>
<tr>
<td>8</td>
<td>CONCLUSIONS</td>
<td>230</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Human milk is considered as nature’s best infant food from nutritional, immunological and food safety points of view (Megraud et al., 1990). However, time constraints, health conditions and urbanization may cause the early termination of breast-feeding. Therefore, there is a need to provide an alternative means of feeding for those infants who cannot have breast-feeding.

Goat milk has been recommended as an ideal substitute for cow milk and has become a more popular alternative infant food (Park, 1994). Goat milk is reportedly less allergenic and more digestible than cow milk (Birkbeck, 1978; Kirke, 1979; Taitz and Armitage, 1984; Park, 1994; Park and Haenlein, 2006). Various authors have reported goat milk to be different from cow or human milk in terms of higher digestibility, higher buffering capacity, and therapeutic values (Gamble et al., 1939; Rosenblum and Rosenblum, 1952; Walker, 1965; Devendra and Burns, 1970; Haenlein and Caccese, 1984; Park and Chukwu, 1988; Park, 1991). Virtually all infants who are allergic to cow milk may be able to tolerate goat milk (Bahner and Heiner, 1980; Park, 1994).

However, goat milk differs from human milk in amino acid composition and casein/lactalbumin ratio, and the protein energy ratio of goat milk is too high for its use as an infant feed (Parkash and Jenness, 1968). Likewise, the fat composition of goat milk is typical of ruminant milks and is a relatively poor source of essential fatty acids (Le Jaouen, 1981; Parkash and Jenness, 1968). Unlike human milk, goat milk is rich in short
and medium chain saturated fatty acids, and deficient in long chain polyunsaturated fatty acids. Human infants also need cholesterol for growth and development including cognitive, visual and neurological functions (Fomon, 1974). The cholesterol content of a typical commercially available infant formula is usually less than one-third of that found in human milk (Forsyth, 1998). Research is, therefore, necessary to develop products that are more nutritionally desirable and homologous to human milk for infant feeding by simulating the macronutrient composition of goat milk to that of human milk.

The overall objective of this study was to produce caprine milk infant formula analogues with nutritional benefits targeted for infant feeding.

Specific objectives were:

- To lower the beta-lactoglobulin content and casein/whey protein ratio of goat milk without change in pH to simulate human milk protein composition.
- To simulate the total fat content, fatty acid profiles and cholesterol content of goat milk to those of human milk for infant feeding.
- To enzymatically produce a structured lipid with similar acyl distribution on the glycerol backbone as in human milk fat, for use in infant milk formulation.
- To characterize the produced structured lipid and determine the effects of antioxidants and long chain polyunsaturated fatty acid supplementation on its oxidative stability during storage.
REFERENCES


Goats rank third after cows and buffaloes in terms of the world’s total annual milk supply. The annual tonnage of milk produced from goats is approximately 2% of the total world milk supply, which is a much smaller quantity compared to cow and buffalo milk production (FAO, 1997; Haenlein, 2006; Park, 2006). On a worldwide basis, however, more people drink the milk of goats than the milk of any other single species (French, 1970; Haenlein and Caccese, 1984; Park, 1990).

During the last 20 years, the total world tonnage of goat milk increased beyond that of sheep milk production (Haenlein, 2006), while there was increased interest for goat milk production and conversion to value-added products. Within continents, Africa leads in goat production relative to all milk produced, while Asia leads in total annual milk tonnage, total goat numbers and relative increase of goat milk production for the past 20 years (Haenlein, 2006).

Europe produces about 18.3% of the world’s goat milk supply, while countries around the Mediterranean region have the most developed dairy goat industries, where France, Greece, Spain and Italy are the main goat milk producing countries in that region (Tzboula-Clarke, 2003). Unlike the cow milk industry, the large-scale industrialization of dairy goat production in many countries is limited due to the low level of milk production (Lowenstein et al., 1980; Juarez and Ramos, 1986).
SIGNIFICANCE OF GOAT MILK IN HUMAN WELLBEING

Although the production volume of goat milk is relatively small in total world milk supply, goat keeping has a significant economic importance in countries where climatic conditions are not favorable for cattle raising (Tzboula-Clarke, 2003; Park, 2006). Due to the unavailability of cow milk, goat milk has become a vital food source of protein, phosphate and calcium in developing countries (Haenlein and Caccese, 1984; Park, 1991) and their contribution to the nutritional and economic wellbeing of mankind is tremendous in many parts of the world (Kosikowski, 1977; Juarez and Ramos, 1986; Park, 1994).

Interest in goat milk stems from the relevant increase in functional food demand and consumption in developed countries, where goat milk is regarded as a substitute for cow milk for individuals having cow milk allergies (CMA) (Walker, 1965; Van der Horst, 1976; Taitz and Armitage, 1984; Park, 1990; Chandan et al., 1992; Park, 1994; Tzboula-Clarke, 2003). In addition, goat milk products have also recently gained increasing popularity among certain ethnic groups, health food lovers, gourmet lovers, goat farmers, and cheese enthusiasts in the United States (Kowsikowski, 1977; Park, 1990). Significant amounts of fluid, evaporated, and powdered goat milk products have been produced and marketed in the United States and New Zealand for many decades (Loewenstein et al., 1980; Park, 1999).

CHEMICAL COMPOSITION OF GOAT MILK

The chemical composition of goat milk varies widely and is influenced by breed, nutritional and environmental factors, stage of lactation, parity, and season of lactation (Park and Chukwu, 1989; Tzboula-Clarke, 2003). On the average, goat milk consists of
12.2% total solids, comprising of 3.8% fat, 3.5% protein, 4.1% lactose and 0.8% ash as shown in Table 2.1.

**Fat in goat milk**

*General properties of goat milk fat*

The lipid fraction of goat milk is relatively high in saturated fatty acids, which is typical of milk fat from other ruminants. The average size of goat milk fat globules is about 3.5 micrometers and is characterized by its high homogeneity which provides lipases with greater surface area of fat for enhanced digestion (Fahmi et al., 1956; Haenlein and Caccese, 1984; Stark, 1988; Chandan et al., 1992). The smaller fat globules found in goat milk allows for better fat dispersion and poor creaming ability of the milk, which provides a natural homogenization that is beneficial to human health (Haenlein and Caccese, 1984; Chandan et al., 1992; Park, 2006).

Reports by Parkash and Jenness (1968), Jenness (1980), Ramos and Juarez (1981) and Ruiz-Sala et al. (1996) reveal that the fat globule membrane of goat milk is composed of the plasma membrane of the secretory cell, phospholipids, cerebrosides, gangliosides and sterols. About 60% of the total phospholipids in goat milk are on the milk fat globule membrane (Tzboula-Clarke, 2003), which includes phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, and sphingomyelin.

Free and bound lipids make-up about 97% and 1-3% respectively of the total fat in goat milk, where the free lipids contain 96.8% triacylglycerols, 2.2% diacylglycerols, and 0.9% monoacylglycerols, while the bound lipids comprise of 46.8%.
Table 2.1: Basic composition of goat milk (Mean values per 100g).

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g)</td>
<td>3.8</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>3.5</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>4.1</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>0.8</td>
</tr>
<tr>
<td>Total solids (g)</td>
<td>12.2</td>
</tr>
<tr>
<td>Calories (cal)</td>
<td>70</td>
</tr>
</tbody>
</table>

Adapted from Park (2006).
neutral lipids and 53.2% polar lipids (Cerbulis et al., 1982; Park, 2006). The polar lipid fraction of goat milk fat consists of 8.5% glycolipids and 44.7% phospholipids (Morrison et al., 1965; Cerbulis et al., 1984). Quantitative analysis of the phospholipid fraction of bound lipids in goat milk by Cerbulis et al. (1982) and Renner et al. (1989) revealed that the phospholipid fraction contained 35.4% phosphatidyl ethanolamine, 3.2% phosphatidyl serine, 4.0% phosphatidyl inositol, 28.2% phosphatidyl choline, and 29.2% sphingomyelin.

**Fatty acids in goat milk**

Goat milk is predominantly composed of triacylglycerols, which consists of one molecule of glycerol to which 3 molecules of fatty acids have been attached. Renner (1982) identified more than 200 fatty acids found in triacylglycerols of milk fat. Fatty acids are generally of different lengths, and can be identified by their number of carbon atoms, which can be saturated or unsaturated, straight chained or branched (Haenlein, 1992; Park, 2006).

Studies by various authors revealed the fatty acid profile of goat milk fat (Table 2.2) is significantly dominated by short and medium chain fatty acids ranging from C4:0-C18:0 (Jenness and Patton, 1976; Jenness, 1980; Jensen et al., 1990; Haenlein, 1992). Goat milk fat is characterized by high contents of C16:0, C8:0, C10:0, and C12:0. Gas liquid chromatographic analysis of goats milk triacylglycerols shows a wide spectrum of molecular weights, while the distribution of acyl carbon numbers shows maxima at C40 and C52 and a minimum at C48 (Ruiz-Sala et al., 1996; Tzboula-Clarke, 2003).
Table 2.2: Fatty acid composition of goat milk fat (g/100g fat).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>2.24</td>
</tr>
<tr>
<td>C6:0</td>
<td>2.43</td>
</tr>
<tr>
<td>C8:0</td>
<td>2.71</td>
</tr>
<tr>
<td>C10:0</td>
<td>9.89</td>
</tr>
<tr>
<td>C12:0</td>
<td>4.89</td>
</tr>
<tr>
<td>C14:0</td>
<td>9.69</td>
</tr>
<tr>
<td>C16:0</td>
<td>30.1</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.59</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.00</td>
</tr>
<tr>
<td>C18:1</td>
<td>18.2</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.33</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Adapted from Park, (2006)
Analysis of the positional distribution of fatty acids in goats milk triacylglycerols by Ruiz-Sala et al. (1996) showed that most of the short chains (C4-C8) fatty acids are esterified at the sn-3 position of the triacylglycerol, while the longer chain (C10-C18) are found at the sn-2 position. According to Tzboula-Clarke (2003), this position implies the synthesis of triacylglycerols from a pool of long-chain 1,2-diacylglycerols in dairy goats.

Alonso et al. (1999) identified some branched chain fatty acids including iso- and anteiso- C15:0, iso- and anteiso- C17:0, and iso- C16:0 in goat milk, which consists of >0.1% of the total fatty acids methyl esters in goat milk. A low content (2.12%) of trans fatty acid has been reported for goat milk fat by Bickerstaffe et al. (1972) and Alonso et al. (1999), of which about 12% of the generic isomers of the oleic acid content was of the trans configuration. In recent years, the consumption of trans fatty acids has been associated with the risk of coronary heart disease (Kris-Etherton, 1995). Elaidic acid has been the basis of most studies relating the intake of trans fatty acids to an increased risk of coronary heart disease (Alonso et al., 1999), and according to Bickerstaffe et al. (1972), elaidic acid (trans-9-C18:1) is the most important isomer of trans-6-C18:1 to trans-9-C18:1 group in goat milk. Free fatty acid content (3.11μeq/ml) of goat milk is reportedly connected with the percent fat and varies with breed and lactation of the animal (Park, 1991; Haenlein 1992; Park 2006).

**Cholesterol in goat milk**

Cholesterol content of goat milk is approximately 11mg/100g of milk, and generally in the range of 10-20mg/100g milk (Posati and Orr, 1976; Jeness, 1980; Park, 2006). The cholesterol in goat milk occurs in a free state with a small fraction (52mg/100g fat) occurring in ester form, which makes up about 4% of the total cholesterol (Jenness, 1980; Chandan et al., 1992). The fatty acid composition of the
cholesterol esters (Table 2.3) reveals a high content of C16:0 (39.3%) and C18:1 (26.5%) (Jenness, 1980; Juarez and Ramos, 1986).

Studies by Arora et al. (1976) show that the level of unsaponifiable matter in goats milk is 24mg/100ml milk or 460mg/100g fat of which most of this lipid fraction (91%) is cholesterol. Cholesterol accounts for about 420mg/100g of goat milk fat with most of this existing in a free state, and a small fraction (52mg/100g) existing in the ester form (Arora et al., 1976). About 66% of the free cholesterol and 42% of the cholesterol ester are present in the goat milk fat globules (Keenan and Patton, 1970). Patton et al. (1980) reported that holding goat milk at refrigeration temperatures increases the phospholipids and cholesterol content in skim milk fraction, as a result of damage to the milk fat globule membranes, which subsequently gets retained in the skim milk during creaming.

**Conjugated linoleic acid**

Conjugated linoleic acid is naturally present in goats’ milk fat and has gained much attention recently due to its beneficial effects on health (Park, 2006). Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid that contain conjugated unsaturated double bonds (Dhiman et al., 1999). Some health benefits associated with these isomers include anticarcinogenic activity (Parodi, 1994; Belury, 1995; Lawless et al., 1998), ability to reduce catabolic effects of immune stimulation (Cook et al., 1993; Lawless et al., 1998), ability to enhance growth promotion (Chin et al., 1994; Lawless et al., 1998), and ability to reduce body fat (Pariza et al., 1996; Lawless et al., 1998). Increased conjugated linoleic acid content in goat milk has been demonstrated through dietary manipulation and supplementation with ingredients such as canola oil (Mir et al., 1999; Park, 2006).
Proteins in goat milk

The principal proteins in goat milk are the same as the milk of other species and are the caseins (κ-, β-, αs1-, αs2-, and γ- caseins) and the whey proteins, β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins (Tzboula-Clarke, 2003). The five major proteins in goat milk are the β-lactoglobulin, α-lactalbumin, κ-casein, β-casein, and the αs2-casein (Carles, 1986, Haenlein and Caccese, 1984, Mikkelsen et al., 1987). Electrophoretic mobility under standard conditions revealed that β-casein is the major component of the casein fraction in goat milk (Park, 2006). The composition of different protein fractions in goat milk is shown in Table 2.3.

Caseins

The αs-caseins in goat milk possess the fastest electrophoretic mobility (Whitney et al., 1976) and are capable of being stabilized by κ-casein against precipitation (Parkash and Jenness, 1968). The major difference between αs1- and αs2-caseins is the disulfide linkage in the former and a complete lack of this linkage or thiol in the latter (Jenness, 1980). Tzboula-Clarke (2003) has shown that polymorphism of αs1-casein controls the level of its excretion, and more than 18 alleles have been identified in goat milk. The alleles are distributed among seven different classes of protein variants (αs1-casein A-G) and are associated with 4 levels of αs1-casein expression. Goat milk has been reportedly lacking in αs1-casein (Aschaffenburg and Dance, 1968; Pierre and Portman, 1970; Jenness, 1980), but recent studies by Mora-Gutierrez (1991) have showed that goat milk may contain different levels of αs1-casein depending on breed of the animal.
Table 2.3: Caseins, minor proteins and enzyme content of goat milk.

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>3.5</td>
</tr>
<tr>
<td>Total Casein (g/100ml)</td>
<td>2.11</td>
</tr>
<tr>
<td>α-s1 (% of total caseins)</td>
<td>5.6</td>
</tr>
<tr>
<td>α-s2 (% of total caseins)</td>
<td>19.2</td>
</tr>
<tr>
<td>β (% of total caseins)</td>
<td>54.8</td>
</tr>
<tr>
<td>κ (% of total caseins)</td>
<td>20.4</td>
</tr>
<tr>
<td>Whey protein (%) (Albumin and Globulin)</td>
<td>0.6</td>
</tr>
<tr>
<td>Alpha-lactalbumin</td>
<td>0.17</td>
</tr>
<tr>
<td>Beta-lactoglobulin</td>
<td>0.43</td>
</tr>
<tr>
<td>Non-protein nitrogen (%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Lactoferrin (µg/ml)</td>
<td>20-200</td>
</tr>
<tr>
<td>Transferrin (µg/ml)</td>
<td>20-200</td>
</tr>
<tr>
<td>Prolactin (µg/ml)</td>
<td>44</td>
</tr>
<tr>
<td>Folate-binding protein (µg/ml)</td>
<td>12</td>
</tr>
<tr>
<td>IgA (µg/ml)</td>
<td>30-80</td>
</tr>
<tr>
<td>IgM (µg/ml)</td>
<td>10-40</td>
</tr>
<tr>
<td>IgG (µg/ml)</td>
<td>100-400</td>
</tr>
<tr>
<td>Lysozyme (µg/100ml)</td>
<td>25</td>
</tr>
<tr>
<td>Ribonuclease (µg/100ml)</td>
<td>425</td>
</tr>
<tr>
<td>Xanthine oxidase (µl O₂/h/ml)</td>
<td>19-113</td>
</tr>
</tbody>
</table>

Adapted from Park (2006).
Various authors report β-caseins are the major component of goat milk casein (Jenness, 1980; Storry et al., 1983; Remeuf and Lenoir, 1986; Renner et al., 1989). According to Whitney et al. (1976), the β-casein of goat milk has more numerous genetic variants than the other caseins, which complicates their electrophoretic differentiation. Park (2006) defined the primary structure of goat milk β-casein with a calculated molecular weight of 23,980. γ-caseins have been shown to be identical with fragments of β-casein through studies involving amino-acid analysis, molecular weight determination, peptide mapping and partial amino-acid sequencing (Groves et al., 1972; Whitney et al., 1976). Dayoff (1979) regard κ-casein as the only component of the goat milk casein for which the entire sequence of amino-acid has been determined. These casein types occur in the form of a mixture of polymers held together by intermolecular disulfide bonds (Swaisgood and Brunner, 1963).

**Whey proteins**

Milk whey proteins include β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, and protease-peptone fractions (Whitney et al., 1976; Jenness, 1980; Park, 2006). Other minor proteins that have been identified in goat milk include lactoferrin, transferrin, prolactin, glycoprotein and folate binding protein (Tzboula-Clarke, 2003; Park, 2006). Goat milk β-lactoglobulin consists of a polypeptide chain of 162 amino-acid residues (Jenness, 1980), and can be identified immunologically by micro complement fraction technique (Alexander and Pace, 1973; Jenness, 1980). The genetic variants of β-lactoglobulin (A, B, C, D) is reported to have originated from point mutations, and differ by substitutions of amino-acids at different positions (Jenness, 1980). The immunoassay of goat milk β-lactoglobulin has shown that it has three less-negatively charged and one more positively charged residues than bovine β-lactoglobulin.
at pH of 5-9, which confers goat milk a relatively slower electrophoresis mostly at alkaline pH levels (Jenness, 1980; Juarez and Ramos, 1986; Park, 2006).

Goat milk α-lactalbumin has no methionine (MacGillivary et al., 1979), and possess immunological cross reactivity with α-lactalbumin from other species milk. Identification of this protein can be done by micro complement fixation technique or by antibody absorption (Priels et al., 1975). Alpha-lactalbumin is required for the biosynthesis of lactose (Ebner and Schanbacher, 1974), and acts as a protein modifier by changing the apparent Km of the substrate (glucose) and does not undergo any reaction changes itself.

Goat milk contains serum albumin, a protein heterogeneous in nature, with a single peptide chain, one free sulfhydryl group at position 34 in the N-terminal peptide, and close to 17 intramolecular disulfide bonds (Whitney et al., 1976). The immunoglobulin G (IgG) content of goat milk is greater in comparison with that of other ruminants (Park, 2006). Using radioimmunoassay, Pashud and Mach (1970) has shown that mature goat milk contains 30 to 80 μg IgA, 10 to 40 μg IgM, and 100 to 400 μg IgG per mL of milk. Immunoglobulins are unique among other milk proteins due to the molecular genetics of their synthesis, their heterogenicity, and their synthesis (Whitney et al., 1976). The nomenclature of immunoglobulins is based primarily on immunochemical criteria, basically on cross reactivity with reference proteins (Park, 2006).

Goat milk contains higher level of non-protein nitrogen (NPN) than cow milk, where NPN is composed of several nitrogenous compounds (Jenness and Patton, 1976; Park, 2006). The true protein was calculated as crude protein minus non-protein nitrogen, and the ratio of casein to true protein in goat milk was 82.7% (Park, 2006).
**Amino acids**

Goat milk proteins revealed a species specific amino acid composition, where differences between casein fractions are much greater than differences between species within a casein fraction (Park 2006). Jenness (1980) and Webb and Johnson (1965) showed that the α-caseins contain greater aspartate, lysine and tyrosine than do β-caseins, while the latter has higher leucine, proline, and valine than the former (Table 2.4). It was also shown that the α-alctalbumin protein contains significantly greater aspartate than does β-lactoglobulin, and vice-versa for alanine and glutamate concentrations (Webb and Johnson, 1965; Jenness, 1980; Park, 2006).

In a comparative study performed by Davis et al. (1994) to determine the amino acid composition of milks among different species of animals, similarities were observed in the overall amino-acid pattern of the milks of all species tested. Park (2006) noted that the most abundant amino acids were glutamate (20%), proline (10%), and leucine (10%). Other commonalities in all species milks revealed that essential amino acids were 40% of the total amino acids, while branched chain amino-acids (BCAA) and sulfur amino-acids were 20% and 4% of the total amino-acids respectively (Davis et al., 1994; Park, 2006).

**Minor proteins**

Goat milk contains about 12 μg/mL of folate-binding protein, which is a glycoprotein with about 22% carbohydrate (Ford et al., 1972; Rubinoff et al., 1977) that binds 9.2 μg folic acid/mg of protein (Jenness, 1980; Park, 2006). Lactoferrin, transferrin, and prolactin content of goat milk amounts to about 20-200μg, 20-200μg, and 44μg respectively per ml of milk (Chandan et al., 1968; Jenness, 1980; Remeuf and Lenoir, 1986; Renner et al., 1989; Park, 2006). Protease-peptones have been reportedly present in
goat milk, and is characterized as a mixture of heat-stable, acid-soluble, phosphoglycol proteins (Rowland, 1937).

**Enzymes**

Ribonuclease in goat milk has been identified as identical to bovine pancreatic ribonuclease (Juarez and Ramos, 1986). Chandan et al. (1968) reported that goat milk contains an average of 25µg lysozyme, 425µg ribonuclease, and 36µml of lipase per 100ml of milk. Alkaline phosphatase content in goat milk ranges form 11-13 mg/l, with inactivation of the enzyme occurring at about 45°C, which indicates that the alkaline phosphatase test may be unsuitable for the assay of adequate heat treatment (Juarez and Ramos, 1968; Park, 2006). Acid phosphatase in goat milk has an activity level of 0.136 units/g of protein (Park, 2006). The molecular weight of acid phosphatase in goat milk has been estimated at 43,000, with 297 residues including 3 of mannose, 1 of galactose and 2 of glucoseamine (Kuzuya et al., 1984).

Xanthine oxidase content of goat milk is relatively low and contains relatively high amounts of aspartic acid, glutamic acid, proline and glycine, and low amounts of serine (Zikakis et al., 1983; Chandan et al., 1992). Juarez and Ramos (1986) associated xanthine oxidase with the control of various redox reactions in the cell, which induces ion absorption, ion oxidation and combination with transferrin, and antibacterial mechanisms via the lactoperoxidase system. The authors also indicated the implication of xanthine oxidase in the off-flavor induction of milk, and development of atherosclerosis in humans.
Table 2.4: Percentage amino-acid composition of isolated proteins of goat milk.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>αs2-casein</th>
<th>β-casein</th>
<th>κ-casein</th>
<th>β-lactoglobulin</th>
<th>α-lactalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>2.80</td>
<td>2.35</td>
<td>9.36</td>
<td>9.88</td>
<td>4.07</td>
</tr>
<tr>
<td>Arg</td>
<td>3.69</td>
<td>1.41</td>
<td>2.92</td>
<td>1.85</td>
<td>0.81</td>
</tr>
<tr>
<td>Asp</td>
<td>7.71</td>
<td>4.23</td>
<td>9.36</td>
<td>8.64</td>
<td>17.89</td>
</tr>
<tr>
<td>Cys</td>
<td>0.81</td>
<td>0.00</td>
<td>1.75</td>
<td>3.09</td>
<td>6.50</td>
</tr>
<tr>
<td>Glu</td>
<td>22.88</td>
<td>20.19</td>
<td>15.20</td>
<td>14.81</td>
<td>10.57</td>
</tr>
<tr>
<td>Gly</td>
<td>0.90</td>
<td>2.82</td>
<td>0.58</td>
<td>3.09</td>
<td>4.07</td>
</tr>
<tr>
<td>His</td>
<td>2.70</td>
<td>2.35</td>
<td>2.34</td>
<td>1.23</td>
<td>2.44</td>
</tr>
<tr>
<td>Ile</td>
<td>4.90</td>
<td>4.23</td>
<td>6.43</td>
<td>6.17</td>
<td>6.50</td>
</tr>
<tr>
<td>Leu</td>
<td>5.34</td>
<td>9.39</td>
<td>4.68</td>
<td>12.96</td>
<td>10.57</td>
</tr>
<tr>
<td>Lys</td>
<td>11.10</td>
<td>5.63</td>
<td>4.68</td>
<td>9.88</td>
<td>10.57</td>
</tr>
<tr>
<td>Met</td>
<td>2.07</td>
<td>2.82</td>
<td>0.58</td>
<td>2.47</td>
<td>0.00</td>
</tr>
<tr>
<td>Phe</td>
<td>4.64</td>
<td>4.23</td>
<td>2.34</td>
<td>2.47</td>
<td>3.25</td>
</tr>
<tr>
<td>Pro</td>
<td>6.88</td>
<td>15.49</td>
<td>11.11</td>
<td>4.94</td>
<td>1.63</td>
</tr>
<tr>
<td>Ser</td>
<td>4.80</td>
<td>7.04</td>
<td>7.60</td>
<td>3.70</td>
<td>4.88</td>
</tr>
<tr>
<td>Thr</td>
<td>5.57</td>
<td>5.63</td>
<td>8.77</td>
<td>4.94</td>
<td>4.88</td>
</tr>
<tr>
<td>Typ</td>
<td>1.47</td>
<td>0.47</td>
<td>0.58</td>
<td>1.23</td>
<td>3.25</td>
</tr>
<tr>
<td>Tyr</td>
<td>7.07</td>
<td>1.88</td>
<td>5.26</td>
<td>2.47</td>
<td>3.25</td>
</tr>
<tr>
<td>Val</td>
<td>4.68</td>
<td>9.86</td>
<td>6.43</td>
<td>6.17</td>
<td>4.88</td>
</tr>
</tbody>
</table>

Adapted from: Webb and Johnson (1965); Jenness (1980); Park (2006).
Lipase is an enzyme that has been implicated in the spontaneous and induced lipolysis of goat milk due to its specific lipolytic system (Chilliard et al., 1984; Haenlein and Caccese, 1984; Park, 2006). Lipase content of goat milk is relatively low and exhibits lower activity at refrigeration temperatures of about 4°C (Chilliard et al., 1984). The lactic dehydrogenase and malic dehydrogenase activity in goat milk has been reported as 47 μmols and 50 μmols respectively per second per ml of milk (Jenness, 1980). In an electrophoretic study performed by this author, goat milk exhibited primarily one lactic dehydrogenase isoenzyme (LDH-1) and one malic dehydrogenase isoenzyme (M-MDH).

**Minerals in goat milk**

The macro-mineral levels of goat milk can vary depending on the breed, diet, animal and stages of lactation (Park and Chukwu, 1988; Park, 2006). Mineral content of goat milk is given in Table 2.5. Goat milk contains about 134 mg calcium and 121 mg phosphorus per 100g of milk. Goat milk serves as an important food source of animal protein, phosphate, and calcium in under-developed countries where cow milk and meat are unavailable (Haenlein and Caccese, 1984; Park, 1991; Park, 1992).

Concentrations of major minerals were reported by various authors, where goat milk is relatively high in calcium, phosphorus, potassium, magnesium and chlorine, and low in sodium and sulfur (Chandan et al., 1968; Posati and Orr, 1976; Jenness, 1980; Haenlein and Caccese, 1984; Debski et al., 1987; Park and Chukwu, 1988, 1989). Studies performed by Konar et al. (1971) and Park and Chukwu (1988) have shown that there is a close inverse relationship between lactose content and the molar sum of sodium
Table 2.5: Mineral composition of goat milk (mg/100g)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>134</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>121</td>
</tr>
<tr>
<td>Magnesium</td>
<td>16</td>
</tr>
<tr>
<td>Potassium</td>
<td>181</td>
</tr>
<tr>
<td>Sodium</td>
<td>41</td>
</tr>
<tr>
<td>Chlorine</td>
<td>150</td>
</tr>
<tr>
<td>Sulfur</td>
<td>2.89</td>
</tr>
<tr>
<td>Iron</td>
<td>0.07</td>
</tr>
<tr>
<td>Copper</td>
<td>0.05</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.032</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.56</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.022</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Adapted from Park (2006).
and potassium contents of goat or other species milk. These authors revealed that chloride is positively correlated with potassium and negatively with lactose, while sodium is neither significantly correlated with potassium, chlorine, nor lactose. According to Maraval and Vignon (1982), major minerals in goat milk during the first seven weeks of lactation fluctuate substantially, where macro minerals decrease in levels with lactation stage: calcium from 1.80-2.00 to 1.23-1.41, magnesium from 0.21-0.27 to 0.10-0.13, phosphorus from 1.43-1.57 to 0.90-0.93, and sodium from 0.43-0.48 to 0.30-0.37 g/l, respectively. Potassium content (1.50-1.80 g/l) was not affected by stage of lactation. The total carbon dioxide and carbonate in fresh goat milk is 3.4mM, with 1.9mM of the carbon dioxide present as bicarbonate ion (Linzell and Peaker, 1971).

Concentrations of trace minerals are affected by diet, breed, animals, and stages of lactation (Park and Chukwu, 1989). Zinc has the highest concentration in goat milk among the trace minerals, while ion concentration is relatively low. Iodine content in goat milk is of relatively high concentration, which may be beneficial to human health, due to the effect of iodine on the thyroid hormone (Underwood, 1977).

**Vitamins in goat milk**

Goat milk contains adequate amounts of vitamin A and niacin, and excess of thiamin, riboflavin, and pantothenic acid with respect to cow milk. Vitamin content of goat milk is given in Table 2.6. Goat milk appears white due to the conversion of β-carotene to vitamin A in the milk (Parkash and Jennness, 1968; Ford et al., 1972). The
Table 2.6: Vitamin content of goat milk

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount (100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (I.U.)</td>
<td>185</td>
</tr>
<tr>
<td>Vitamin D (I.U.)</td>
<td>2.3</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.068</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.21</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.27</td>
</tr>
<tr>
<td>Pantothenic acid (mg)</td>
<td>0.31</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>0.046</td>
</tr>
<tr>
<td>Folic acid (μg)</td>
<td>1.0</td>
</tr>
<tr>
<td>Biotin (μg)</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>0.065</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Adapted from Park (2006).
vitamin B level in goat milk is as a result of rumen synthesis, and is independent of diet (Haenlein and Caccesse, 1984). Goat milk is reportedly deficient in folic acid, vitamin B<sub>12</sub> (Colins, 1962; Davidson and Townley, 1977; Jenness, 1980; Haenlein and Caccesse, 1984; Park et al., 1986). Vitamin B<sub>12</sub> deficiency has been implicated in goat milk anemia, and folate is necessary for the synthesis of hemoglobin (Collins, 1962; Davidson and Townley, 1977). However, the major cause of anemia has been attributed to the folate deficiency in goat milk (McLennan and Walker, 1982).

PHYSICO-CHEMICAL CHARACTERISTICS OF GOAT MILK

*Physico-chemical properties of goat milk*

The physico-chemical properties of goat milk are shown in Table 2.7. The saponification value and refractive index of goat milk reflects the number of carbons and saturation in the milk fatty acid. Goat milk has high Reichert Meisl value and Polenske value, suggesting that goat milk fat contains high soluble volatile fatty acids (Park, 2006). Correlations exist between the chemical structures and viscosity of milk, whereby the microstructures controls physico-chemical properties of milk such as viscosity, elasticity, texture and firmness (Lan et al., 2000). Viscosity is a major physical property of goat milk, and has crucial effects on its flow characteristics (Saif et al., 2004). Protein aggregation, gelation, and sedimentation are highly influenced by the viscosity and pH of goat milk. The viscosity of goat milk range from 4.40-4.61 centipoises, which is significantly higher than the 2.12 centipoises reported by El-Agmy (1983). The pH range
Table 2.7: Physico-chemical properties of goat milk

<table>
<thead>
<tr>
<th>Properties</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsaponifiable matter of milk fat</td>
<td>0.41</td>
</tr>
<tr>
<td>Acid value</td>
<td>0.47</td>
</tr>
<tr>
<td>Iodine Value</td>
<td>30.44</td>
</tr>
<tr>
<td>Saponification value</td>
<td>228.6</td>
</tr>
<tr>
<td>Reichert Meissl value</td>
<td>29.16</td>
</tr>
<tr>
<td>Polenske value</td>
<td>1.80</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.450</td>
</tr>
</tbody>
</table>

Adapted from Ajeneyulu et al., (1985); Park (2006).
of goat milk is in the range of 6.56-6.59 (Maduko and Park, 2004; Maduko et al., 2004a,b).

Fat in goat milk occurs as small droplets of different diameters (average 3.5 μm), which provides naturally homogenized fluid milk (Park, 2006). Goat milk fat is solid at room temperature, and has a melting point of 37°C, while the melting point of its individual triacylglycerol ranges from –75°C to 72°C (Wong et al., 1996). Solid concentration level, temperature, shear rate, and presence of various particulate compositions, have been observed to have an effect on the viscosity of milk (Lan et al., 2000; Lange, 1967; Kessler, 1981; Kubota, 1980; Phipps, 1969). According to Velez-Ruiz and Barbosa-Carnovas (1997) and Lan et al. (2000), fat content is one of the most important factors affecting the rheological properties of goat milk. Other physical properties of goat milk fat includes its density of 915 kg/m³ at 20°C, refractive index of 1.462 at 589nm, thermal conductivity of 0.17 J/m/s/K at 20°C, specific heat capacity of 2.1KJ/kg/K at 40°C, electric conductivity of about 10-12/ohm/cm, and a dielectric constant of about 3.1 (Walstra and Jenness, 1984).

Optical and color properties of goat milk

Fresh goat milk is a white opaque liquid with a slightly sweet taste and has no odor (Le Jaouen, 1987). Color and appearance are major quality attributes for the acceptability of goat milk products. Goat milk color depends on fat and solid contents, which are affected by feed, breed, physiological and lactation stages of the animals (Maduko et al., 2005a; Haenlein, 2006; Park, 2006). Fresh goat milk has an extremely white color that is due to the absence of beta-carotene in the milk fat. Goat milk also has
fat globules, which disperses light and induces a white appearance to the milk. Skim goat milk has a bluish-gray appearance that is due mainly to its content of casein micelles (LeJaouen, 1987; Maduko et al., 2005a; Haenlein 2006). Goat milk whey has a characteristic greenish tint, which is attributed to its high riboflavin content (Maduko et al., 2005a). Optical properties of goat milk provide the basis for indirect proximate content analysis. The refractive index of goat milk is in the range of 1.344 to 1.348 and is used to estimate the total solids content of the milk (Wong et al., 1996).

HYPOALLERGENIC AND THERAPEUTIC PROPERTIES OF GOAT MILK

Allergenic properties of cow milk

Food consumption presents the body with numerous antigens capable of causing an immunologic reaction (Park, 1994). Food allergy results from sensitization of an individual to dietary proteins or other food allergens existing in the intestinal lumen (Firer et al., 1981; McClenathan and Walker, 1982; Heyman and Desjeux, 1992). Cow milk allergy (CMA) is a frequent disease in infants and is mostly caused by β-lactoglobulin, a major whey protein that is absent in human milk (Heyman et al., 1990; Park, 1994). Bleumink and Young (1968), Goldman et al. (1963), and Wahn and Ganster (1982), have shown that αs1-casein, β-lactoglobulin and α-lactalbumin are the major allergens in cow milk. Heyman et al. (1990), Park (1994), Taylor (1985) listed the clinical symptomology for patients allergic to cow milk proteins as: rhinitis, diarrhea, vomiting, asthma, anaphylaxis, urticaria, eczema, chronic catarrh, migraine, colitis and epigastric distress. Several authors indicated that sensitivity to cow milk may be due to α-lactalbumin, which is species specific, and β-lactoglobulin (Zeyman, 1982; Heyman and Desjeux, 1992; Park and Haenlein, 2006).
The α-lactalbumin from goat milk shows a different skin reaction in comparison to cow milk (Park and Haenlein, 2006). Perlman (1977) reported variation in skin test reactions to allergenic fractions of cow milk and goat milk, where results of this study showed that cow proteins have higher incidences of positive skin test reactions than goat milk proteins (Park and Haenlein, 2006).

*Therapeutic and hypoallergenic capacity of goat milk*

Compared to cow milk, goat milk is reported to possess unique characteristics, such as high digestibility, distinct alkalinity, high buffering capacity, and certain therapeutic values (Gamble et al., 1939; Rosenblum and Rosenblum, 1952; Walker, 1965; Devendra and Burns, 1970; Haenlein and Caccesse, 1984; Park, 1991, 1994; Park and Haenlein, 2006). Because of these therapeutic and hypoallergenic properties, goat milk has been recommended by many authors as an alternative to cow milk for patients who suffer from CMA (Rosenblum and Rosenblum, 1952; Walker, 1965; Taitz and Armitage, 1984; Park, 1994; Park and Haenlein, 2006).

Walker (1965) reported that only one in 100 infants who were allergic to cow milk did not thrive well on goat milk. In extensive clinical studies performed in France with CMA children, treatment with goat milk produced positive results in 93% of the cases and was recommended for infant nutrition due to its less allergenicity and better digestibility advantages over cow milk (Fabre, 1997; Grzesiak, 1997; Reinert and Fabre, 1997). In another study, Brenneman (1978) reported that about 40% of CMA patients can tolerate goat milk proteins. Bevilacqua et al. (2000) also showed that the treatment of goat milk therapy on CMA infants resolved 30-40% of the cases, while 49-55% of CMA children benefited from goat milk feeding.
Goat milk produces a softer, more friable curd than does cow milk because goat milk is devoid of αs1-casein, a major protein in cow milk (Coveney and Darton-Hill, 1985), which makes it more digestible than cow milk. According to Ambrosoli et al. (1988), goat milk with the genetic trait of low or no αs1-casein, has less curd yield, longer rennet coagulation time, more heat lability, and weaker curd firmness, which is also responsible for its digestive benefits (Park and Heanlein, 2006). Several authors also report positive response of goat milk therapy on infants suffering from gastrointestinal allergy and clinical enteropathy induced by cow milk consumption, although an immunological cross reactivity exists between goat milk and cow milk proteins (Rosenblum and Rosenblum, 1952; Walker, 1965; Firer et al., 1981; Park 1994; Park and Haenlein, 2006).

According to Rosenblum and Rosenblum (1952), gastrointestinal allergy in certain infants with eosinophilia improved after administration of goat milk. Maszewska-Kuzniarz and Sonta-Jakimaczyke (1973) reported that a chronic enteropathy induced by cow milk formula was healed by goat milk substitution. Van der Horst (1976) also reported successful management of cow milk allergy by substitution with goat milk formula. However, no untreated goat milk should ever be fed to young babies because of the risk of bacterial infection (Taitz and Armitage, 1984). The spray-dried powder is less likely to carry any risk of infection, but its composition is unsatisfactory as an infant formula for infants under 6 months of age (Taitz and Armitage, 1984). Its solute load is high - the level of sodium, potassium, and other electrolytes is similar to that of cow milk (Taitz and Byers, 1972; Taitz and Armitage, 1984). Heat denaturation in evaporated goat milk alters the basic protein structure and thereby decreases the allergenicity and
HUMAN MILK

CHEMICAL COMPOSITION OF HUMAN MILK

Proteins in human milk

Human milk is a low-protein food, with a protein energy ratio of 7% (Morgan, 2006). The energy, macronutrient and selected micronutrient content of human milk are listed in Table 2.8. The principal nutritional function of human milk protein is to meet physiological needs of the human infant, by supplying adequate amounts of dietary essential amino acids, and total nitrogen (Koletzko et al., 2005).

The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) coordinated international expert group calculated the mean amino acid content of human milk (Table 2.9). Human milk protein contains insoluble casein and soluble whey proteins in the ratio of 40% casein to 60% whey protein, which is unlike the 70% casein to 30% whey protein content of goat milk.

The casein protein in human milk is associated with phosphate, magnesium, and citrate ions, which are bound together as a calcium caseinate complex (Morgan, 2006). These caseins form small micelles with a loose structure, which facilitates specific action during digestion (Coveney and Darton-Hill, 1985). Approximately 25% of total human milk nitrogen exists as non-protein nitrogen (NPN), where 50% of NPN is urea, with other compounds in small quantities such as glucoseamines, nucleotides, free amino acids, polyamines, and biologically active peptides (Morgan, 2006).
Table 2.8: Energy, macronutrient and selected micronutrient content of human milk (per 100 ml).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Human milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>67</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>4.2</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>7.0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>15</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>35</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.3</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Retinol (μg)</td>
<td>60</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>4</td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>5</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.02</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>Trace</td>
</tr>
</tbody>
</table>

Adapted from Morgan (2006).
Table 2.9: Amino-acid content of human milk

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Mean Content (mg/g nitrogen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>196</td>
</tr>
<tr>
<td>Cystine</td>
<td>131</td>
</tr>
<tr>
<td>Histidine</td>
<td>141</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>319</td>
</tr>
<tr>
<td>Leucine</td>
<td>586</td>
</tr>
<tr>
<td>Lysine</td>
<td>395</td>
</tr>
<tr>
<td>Methionine</td>
<td>85</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>282</td>
</tr>
<tr>
<td>Threonine</td>
<td>268</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>114</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>259</td>
</tr>
<tr>
<td>Valine</td>
<td>315</td>
</tr>
</tbody>
</table>

Adapted from Koletzko et al., (2005).
Fats in human milk

Human milk fat contributes about 53% of the fat to energy ratio in the milk. The lipids in human milk are triacylglycerol (98%), cholesterol (0.4%), and phospholipids (1.3%) (Jensen, 1989; Jensen et al., 1990). Lipid content of human milk varies with duration of lactation (Dewey et al., 1984; Jensen, 1989), with time of day (Hall, 1979; Harzer et al., 1983; Jensen, 1989), and from start to finish of an individual diet (Hytten, 1954; Hall, 1979; Jensen, 1989; Koletzko et al., 1992a).

Fatty acids

More than 20 fatty acids have been identified in human milk, with seven fatty acids representing 90% of total fat as oleic, palmitic, lauric, linoleic, myristic, stearic, and capric acids (van Beusekom et al., 1993). The fatty acid profile of human milk is presented in Table 2.10. Human milk triacylglycerols (TAGs) are mainly composed of long chain fatty acids with medium chain fatty acids contributing only about 7% of the total fat. Saturated fats contribute about 50.1%, while unsaturated fats account for 48.5% of total lipid in human milk (British Department of Health and Social Security, 1981; Forsyth, 1998). The fatty acid composition of the TAG in human milk can vary with maternal diet, length of gestation, duration of lactation, and parity (Prentice et al., 1989; Koletzko et al., 1988).

Human milk contains a high proportion of long-chain polyunsaturated fatty acids (PUFAs) in response to the needs of the developing brain and nervous system, ex-utero (Morgan, 2006). Human milk fat is high in unsaturated fatty acids, particularly the essential fatty acids, linoleic (C18:2ω6) and α-linolenic acid (C18:3ω3). Studies conducted in Europe, Africa and Australia, have demonstrated that levels of essential
PUFAs in human milk are influenced by the maternal diet of the lactating women, while the other long chain polyunsaturated fatty acid concentrations are less affected (Gibson and Keenbone, 1981; Koletzko et al., 1988, 1992a).

Westermark and Antila (2000) have shown that arachidonic acid (C20:4ω6) is supplied in sufficient amounts to support the structure and function of neural and brain tissue in fetal life and in postnatal development. Long chain PUFAs have been reportedly important in brain development and visual acuity of infants, where pre-term infants may have difficulty in synthesis of long-chain PUFAs from their precursors in sufficient quantities (Morgan, 2006). Moreover, the content of ω-6 and ω-3 long chain PUFAs in human milk is not related to the availability of respective precursors - linoleic and α-linolenic acid (Gibson and Keenbone, 1984; Koletzko et al., 1988; Forsyth, 1998; Morgan, 2006). In contrast, ω-6 and ω-3 long chain PUFAs are correlated with each other, which may reflect a protective metabolic mechanism that provides the breast-fed infant with a balanced ratio of the two long chain PUFA (Koletzko, 1992; Forsyth, 1998). However, reports by Gibson and Keenbone (1981); Harris et al. (1984); Keenbone et al. (1985); Carlson et al. (1986); Innis and Kuhnlein (1988); Koletzko et al. (1988); Innis et al. (1990); Sanders and Ready (1992) have revealed that if the dietary intake of preformed ω-3 PUFA is significantly increased, it would be reflected by an increase in the ω-3 long chain PUFA concentration in the milk (Forsyth, 1998). More recently, a direct correlation has been demonstrated between the level of docosahexaenoic acid (DHA) in human milk and DHA in the maternal diet (Makrides et al., 1996; Forsyth, 1998). Reviews by Forsyth (1998) and Wroble et al. (2002), concluded that ω-3 long chain PUFA supplementation may give rise to early visual maturation in pre-term infants, although the long term benefits of this practice have not been documented.
Table 2.10: Fatty acid profile of human milk

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Amount (g/100g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0</td>
<td>2</td>
</tr>
<tr>
<td>C12:0</td>
<td>7</td>
</tr>
<tr>
<td>C14:0</td>
<td>8</td>
</tr>
<tr>
<td>C16:0</td>
<td>23</td>
</tr>
<tr>
<td>C16:1ω9</td>
<td>3</td>
</tr>
<tr>
<td>C18:0</td>
<td>7</td>
</tr>
<tr>
<td>C18:1ω9</td>
<td>38</td>
</tr>
<tr>
<td>C18:2ω6</td>
<td>9</td>
</tr>
<tr>
<td>C18:3ω3</td>
<td>1</td>
</tr>
<tr>
<td>C20:1ω9</td>
<td>1</td>
</tr>
<tr>
<td>C20:4ω6</td>
<td>0.8</td>
</tr>
<tr>
<td>C22:1ω9</td>
<td>Trace</td>
</tr>
</tbody>
</table>

Data obtained from USDA (1976).
Cholesterol

Human milk has a high level of cholesterol, which is metabolized for growth, cognitive and visual development, and neurological functions for the human infant (Fomon, 1974). Cholesterol is synthesized in the plasma and mammary gland, and the extent of synthesis is hardly affected by diet (Jensen, 1989). The sterol content of human milk is shown in Table 2.11. Cholesterol is not an essential dietary nutrient since the human infant can synthesize it endogenously. The probable long-term effects of infant formula on blood sterol levels have been of great interest in the last decade, stemming from suggestions that infant feeding methods may influence the risk of cardiovascular problems in later life (Pitkin et al., 1972). Breast-fed infants unlike formula-fed infants, develop higher total plasma cholesterol levels, higher plasma low-density lipoprotein-to-cholesterol concentrations, and a higher low-density lipoprotein to high-density lipoprotein ratio (Jooste et al., 1991; Hayes et al., 1992; Kallio et al., 1992). According to Mellies et al. (1976), infants fed phytosterol rich diets had substantially elevated blood plasma phytosterols, unlike breast-fed infants.

Lactose in human milk

Lactose accounts for 80% of the carbohydrates in human milk and for about 40% of its total energy. Lactose may provide beneficial effects for gut physiology, including pre-biotic effects, softening of stools, and enhancement of water, sodium, and calcium absorption (Koletzko et al., 2005). Other carbohydrates in human milk include monosaccharides, oligosaccharides, and protein-bound carbohydrates (Weaver and Prentice, 2003).
Table 2.11: Sterol distribution of human milk.

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Amount (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>14</td>
</tr>
<tr>
<td>Campesterol</td>
<td>0.9*</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0.97*</td>
</tr>
<tr>
<td>Beta-sitosterol</td>
<td>1.71*</td>
</tr>
<tr>
<td>Other sterols</td>
<td>2*</td>
</tr>
</tbody>
</table>

* Calculated from total of ‘phytosterols’ in human milk.

Data obtained from Jensen (1989).
COMPOSITIONAL DIFFERENCES BETWEEN HUMAN AND GOAT MILK

The most remarkable difference in basic composition between human milk and goat milk exists in protein and ash contents, where human milk has substantially lower levels of the two components than goat milk (Park, 2006). However, differences in total solids and caloric values between human and goat milks are not significant (Posati and Orr, 1976; Jenness, 1980; Haenlein and Caccesse, 1984; Park, 2006). According to Jenness (1980), the prominent difference is in the proportion of energy derived from lactose and protein. The nutritional composition of goat and human milk is compared in Table 2.12.

Although goat, cow and human milks are approximately iso-energetic, lactose and protein supply different proportions in the total energy content. The fractions of the energy supplied by fat, protein and lactose in cow and goat milk are 50, 25, and 25% respectively, in comparison to 55, 7 and 38% in human milk (Jenness, 1980). Fatty acids contribute to the structure and function of cellular membranes and influence vital physiological and metabolic processes of the growing infant (Mohrhauer and Holman, 1963a,b; Clandinin et al., 1989; Hernell, 1990; Innis, 1991). Human milk fat (HMF) consists of 98% triacylglycerols (TAG) and 0.4% cholesterol (Jensen et al., 1990). Total human milk TAG is composed mainly of long chain fatty acids, with about 50% of saturated fatty acids and 48.5% of unsaturated fatty acids (British Department of Health and Social Security, 1981).

Human milk contains about 4.4% total lipids, and HMF contains mostly long chain fatty acids such as palmitic, oleic, linoleic and stearic acids. Unlike in vegetable
Table 2.12: Nutritional composition of goat and human milks (per 100g).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Goat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>87.5</td>
<td>87.4</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>71</td>
<td>69</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Casein: Whey</td>
<td>70:30</td>
<td>40:60</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>4.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Adapted from Jenness (1980).
oils and ruminant milk fats, palmitic acid in HMF constitutes the highest proportion (53-70%) of saturated fatty acids at the sn-2 position of the triacylglycerol (TAG) backbone, and unsaturated fatty acids at the sn-1 and sn-3 positions (Jensen, 1989; Innis et al., 1995; and Xu, 2000). Insoluble calcium and fatty acid complexes (calcium soaps) can be formed from free long chain saturated fatty acids released by pancreatic lipase hydrolysis from sn-1 and sn-3 positions of the TAGs of ruminant milk fats and vegetable oils. These calcium soaps may result in low fatty acid and calcium absorption in the lumen of infants as well as loss of dietary energy, stool hardness and in some cases, bowel obstruction (Quinlan et al., 1995; Kennedy et al., 1999). The location of palmitic acid at the sn-2 position of TAG in HMF increases the absorption of palmitic and stearic acids in the lumen of infants and decreases the loss of calcium in their feces (Quinlan et al., 1995; Kennedy et al., 1999). This is due to the preservation of the sn-2 positional palmitic acid during digestion, absorption and biosynthesis of TAGs in the intestinal wall.

Goat milk has reportedly lower (11mg/100g) content of cholesterol than human milk (14mg/100g) (Posati and Orr, 1976; Park, 2006), where the low cholesterol value in goat milk may be of importance to human nutrition, since cholesterol is associated with coronary heart diseases (Park, 2006). Unlike goat milk, human milk also contains significant levels of phytosterols including campesterol, stigmasterol, and beta-sitosterol (Jensen, 1989).

Human milk differs from goat milk in both amino acid composition and casein: whey ratio, where the protein energy ratio of goat milk is too high for its’ use as an infant feed (Parkash and Jenness, 1968). Goat milk has low levels of αs1-casein (0.12g/l to 2.7g/l) (Park, 2006) and high levels of αs2-casein (19.2g/l), where both casein proteins are completely lacking in human milk.
Beta-lactoglobulin is a milk protein highly resistant to intestinal luminal hydrolysis and mostly responsible for milk allergy (Heyman and Desjoux, 1992). This protein is a major whey protein in goat milk and is completely lacking in human milk (Park, 1994). Goat milk β-lactoglobulin like its cow milk homolog consists of a polypeptide chain of 162 amino acid residues (Jenness, 1980). The removal of β-lactoglobulin from milk is difficult due to its solubility at normal milk pH (Park and Haenlein, 2006). Furthermore, goat milk β-lactoglobulin has 3 less-negatively charged and one more positively charged residue than bovine β-lactoglobulin at pH of 5 to 9. Elimination of β-lactoglobulin from goat milk is therefore one step toward humanizing the protein composition of goat milk for infant feeding.

CONSIDERATION OF SUITABILITY OF GOAT MILK IN INFANT NUTRITION

The suitability of goat milk for infant feeding stems from its closeness to human milk, as well as its hypoallergenic and therapeutic values for the infants suffering from cow milk allergy (CMA). Goat milk is becoming a more popular food for infants, where there has been an increased availability of fluid, evaporated and spray-dried goat milk products within the past decade (Birkbeck, 1978; Kirke, 1979; Taitz and Armitage, 1984; Coveney and Darton-Hill, 1985; Haenlein, 2006; Park, 2006).

Despite its nutritional benefits, the chemical composition of goat milk shows lack of proper attributes for infant feeding, since goat milk has greater similarity to cow milk than human milk with respect to amino acid composition and casein:whey ratio (Parkash and Jenness, 1968; Coveney and Darton-Hill, 1985; Park, 2006). The protein-energy ratio of goat milk is too high for its use as an infant feed and might lead to an oversupply of amines, which will subsequently increase the urea level of the blood and urine of infants.
(Coveney and Darton-Hill, 1985). The fat composition of goat milk is typical of ruminant milks and is a relatively poor source of essential fatty acids (Parkash and Jennes, 1968; Park, 2006). Furthermore, the distribution of fatty acids on the TAG backbone of goat milk is unlike that of human milk and may lead to inefficient fatty acid and calcium absorption in the lumen of infants, as well as loss of dietary energy, stool hardness and bowel obstruction (Kennedy et al., 1999; Quinlan et al., 1995).

Goat milk contains high levels of certain minerals and if fed to infants, may lead to risk of hypertonic dehydration (Taitz and Armitage, 1984; Coveney and Darton-Hill, 1985). The high potassium and chloride of goat milk might lead to acidosis in infants (Harrison et al., 1979), while the high calcium:protein ratio of goat milk relative to human milk may cause neonatal tetany and hypercalcaemia in infants (Taitz and Armitage, 1984). Goat milk is reportedly deficient in folic acid, vitamins B6, B12, C, and D (Jenness, 1980; Park, 2006). Braude (1972), Davidson and Townley (1977), and Johnson (1982) reported several cases of megaloblastic anaemia and convulsions in infants who were fed unfortified goat milk.

It is, therefore, necessary to develop goat milk products that are nutritionally desirable and homologous to human milk for infant feeding by simulating the nutrient composition of goat milk to that of human milk (Kirke, 1979; Taitz and Armitage 1984; Coveney and Darton-Hill, 1985; Quinlan et al., 1995; Kennedy, 1999; Park, 2006).

INFANT FORMULA AS AN ALTERNATIVE MEANS OF INFANTS’ DIET

NECESSITY OF INFANT FORMULAS

The recommended diet for newborn infants is human milk (American Academy of Pediatrics, 1976; Fomon, 1986, 1993). Human milk is also considered best for infants
from nutritional and immunological points of view, as well as from the standpoint of protection against harmful bacteria (Megraud et al., 1990).

Nevertheless, time constraints and urbanization may cause the early termination of breast-feeding. Also, some infants are not breast-fed due to short supply of breast milk, insufficient nutrition and health conditions of nursing mothers, death of mothers during or after childbirth, and the necessity of some mothers having to work. Therefore, there is a need for an alternative means of feeding for infants who do not have availability or accessibility to human breast milk.

As a demand for an alternative to breast milk continues, a human milk substitute should, as closely as possible, meet the nutritional requirements of the infants (Forsyth, 1998). Therefore, the ultimate goal in the design of human milk substitutes should be the achievement of the same outcome as in breast-fed infants (British Department of Health, 1996). Infant formulas, developed to replace or supplement human milk should be modified with the goal to be as close as possible to human milk in composition and physiological benefits (Rudolf and Kunz, 1997; Lien, 1994). According to Packard (1982), most infant formulas presently available in the United States are adaptations of the product designed in the last century. Over the years, the composition of infant formulas have been altered and adjusted, mostly in response to scientific evidence of need (Packard, 1982). Infant formulas have been categorized into four major types by Packard (1982), of which the milk-based formula is the most common, and consists mainly of milk ingredients, vegetable oils, and either lactose or corn syrup solids as a carbohydrate source.
COMPOSITIONAL STANDARDS FOR INFANT FORMULAS

The codex standard on infant formula adopted in 1981 was based on the scientific knowledge available in the 1970’s, and is currently under revision (Codex Alimentarius 1987). As part of the revision process, the ESPGHAN committee on nutrition, under direction from the Codex Committee on Nutrition and Foods for Special Dietary Uses, has recently proposed a global standard for the composition of infant formula. According to the committee (Koletzko et al., 2005), the composition of infant formula should meet particular requirements, and promote normal growth and development of the infants for whom they are intended. The committee concluded that infant formula should only contain components in such amounts that serve a nutritional purpose or provide additional benefits. The committee further recommended that infant formula should contain between 60 and 70 kcal of energy per 100ml, as well as macronutrients that are within the levels listed in Table 2.13.

Protein source for milk-based formulas is obtained from skim milk and includes major proteins of casein and whey. The fat of infant formula may stem from a number of oil sources. Vegetable oils such as coconut oil, safflower oil, and soybean oil, when combined in specific proportions, can be used as a suitable source of fat in infant formulas (Packard et al., 1982; Maduko et al., 2005b; 2006a,b,c). Coconut oil is a relatively saturated fat that is rich in medium- and long-chain saturated fatty acids, while safflower and soybean oils are good sources of polyunsaturated fatty acids.

Although long-chain polyunsaturated fatty acids (PUFAs) supplementation is currently under debate in the United States, Canada, and Great Britain, many countries in Asia and Europe mandate the addition of certain long chain PUFAs to infant formula (Wroble et al., 2002). Many controversies surround the addition of eicosapentaenoic acid
and docosahexaenoic acid to infant formula, due to beneficial claims including: prevention of mental deficiencies; increase in brain development; and improvement of early vision acuity. Potential liabilities also attributed to PUFA supplementation in infant formulas include: retardation of physical growth and increased susceptibility to infectious diseases (Hoefer and Hardy, 1929; Rogerson and Rogerson, 1939; Rogers et al., 1978; McCall, 1979; Kopp and McCall, 1982; Taylor and Wadsworth, 1984; Life Science Research Office, 1988; Morely et al., 1988; Uauy et al; 1990; Birch et al., 1992; Lucas et al., 1992, 1994; Jacobsen and Jacobsen, 1992; Carlson et al., 1993, 1996; McCall and Carriger 1993; Innis et al., 1995; Auested et al., 1995; Gibson et al., 1996; Courage et al., 1998; Scott et al., 1998; Willatts et al., 1998; Wroble et al., 2002; Scientific Committee on Food, 2003). The ESPGHAN Committee on Nutrition views the addition of these long chain PUFAs as optional in infant formula.

The successful production of infant milk fat containing PUFAs can be impeded by the high susceptibility of these fatty acids to oxidative deterioration (Let et al., 2005). PUFAs contain several double bonds within their carbon chain that makes them readily susceptible to oxidation (Sahin et al., 2006; Maduko et al., 2006d,e). These fatty acids are broken down into primary products (hydroperoxides) during peroxidation, after which the hydroperoxides are readily decomposed to form complex mixtures of secondary oxidation products like alkanes, alkenes, aldehydes, and ketones (Akoh and Moussata, 2001; Akoh, 2002; Osborn and Akoh, 2004; Romeo-Nadal et al., 2004). Oxidation can alter the flavor and nutritional quality of lipids and produce toxic compounds, which make the oils less acceptable or unacceptable for consumption (Akoh, 2002; Fomuso et al., 2002). Oxidation products typically include low molecular weight volatile compounds as well as undesirable off-flavor compounds. Infant formulas containing
Table 2.13: Proposed macronutrient composition of infant formula by ESPGHAN Committee on Nutrition (2005).

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>kcal/100ml</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/100kcal</td>
<td>1.8</td>
<td>3</td>
</tr>
<tr>
<td>Total fat</td>
<td>g/100kcal</td>
<td>4.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>g/100kcal</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>α-linolenic acid</td>
<td>mg/100kcal</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>Linoleic: α-linolenic acid ratio</td>
<td></td>
<td>5:1</td>
<td>15:1</td>
</tr>
<tr>
<td>Lauric and myristic acid total</td>
<td>% of Total Fat</td>
<td>NS</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>g/100kcal</td>
<td>9.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

NS: Not specified

Adapted from Koletzko et al., 2005.
many unsaturated fatty acids especially long chain PUFAs are very susceptible to oxidation (Let et al., 2005; Maduko et al., 2006d,e; Nielsen et al., 2006).

REQUIRED PROCEDURES FOR DEVELOPMENT OF INFANT FORMULAS

SIMULATION OF INFANT FORMULA COMPOSITION TO HUMAN MILK

The production of infant formula from ruminant milk necessitates the need to alter the naturally occurring macronutrient composition of the milk, in order to lower protein content without loss of biological quality and to modify fat composition of the milk. The micronutrients such as minerals and vitamins also have to be simulated. Skim milk and vegetable oils are usually used for infant milk production. The skim milk provides the caseins and lactalbumin, whereby the ratio of the two proteins must be adjusted to resemble that of human milk. The vegetable oils must also be combined in proportions that would deliver the same fatty acid profiles and total fat of human milk.

A number of relatively new technologies that find application in infant formula processing includes membrane processing and enzymatic transesterification processes.

SIMULATION PROCEDURES FOR MILK PROTEINS AND FATS

Simulation of milk proteins

The need for milk protein modification

Milk contains on average 3.5g per 100g proteins, approximately 75% of which are caseins, with the remainder being the whey proteins. The caseins are insoluble at pH 4.6 and 20°C and are organized in milk in the form of large spherical micelles (Zydney, 1998; Park, 2006). The whey protein content of milk contains a wide array of soluble
proteins with the main components summarized in Table 2.14. The casein:whey protein ratio of human milk is markedly different from ruminant milks, and to humanize this ratio, some of the available commercial infant formulas are fortified with whey proteins (Packard, 1982; Kuwata et al., 1985). However, this practice alone does not minimize some of the compositional differences between the milk of different species such as: higher content of lactalbumin, lactoferrin, lysozyme and immunoglobulins A, in human milk than in milk of other species (Kuwata et al., 1985; Hambraeus, 1997; Park, 2006).

Beta-lactoglobulin is a milk protein that is completely lacking in human milk. It is highly resistant to intestinal luminal hydrolysis and mostly responsible for milk allergy (Robertson et al., 1982; Taylor, 1985; Heyman and Desjeux, 1992; Park, 1994; Park, 2006; Park and Haenlein, 2006). While β-lactoglobulin is the dominant whey protein in milk of ruminants accounting for up to 60% of the total whey proteins, α-lactalbumin and lactoferrin are predominant in human milk whey at levels of 40 and 25% respectively (Liberatori and Napolitano, 1980; Wharton, 1981; Kuwata et al., 1985; Tzboula-Clarke, 2003; Morgan, 2006; Park, 2006). Elimination of β-lactoglobulin from milk is therefore one step towards humanizing the protein composition of milk for infant feeding (Kuwata et al., 1985; Maduko et al., 2005c). However, the removal of β-lactoglobulin from milk is difficult due to its pH dependency in the complex biological fluid system (Juarez and Ramos, 1986; Tolkach and Kulozik, 2005; Park, 2006). The wide-ranging potential applications of the individual whey proteins have generated a lot of work on whey protein fractionation. However, none of the existing techniques has been effectively implemented at commercial scale (Zydney, 1998).
Table 2.14: Characteristics of major whey proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight (g/mol)</th>
<th>Isoelectric pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactoglobulin (monomer)</td>
<td>18,362</td>
<td>5.2</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>14,147</td>
<td>4.5-4.8</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>150,000-1,000,000</td>
<td>5.5-8.3</td>
</tr>
<tr>
<td>Bovine Serum Albumins</td>
<td>69,000</td>
<td>4.7-4.9</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>78,000</td>
<td>9</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>89,000</td>
<td>9.5</td>
</tr>
<tr>
<td>Glycomaropeptide</td>
<td>7,000</td>
<td></td>
</tr>
</tbody>
</table>

Data from Wong et al., (1996); Cayot and Lorient (1997).
**Modification and fractionation of whey proteins**

Most previous studies on whey fractionation have focused on ion exchange or affinity chromatography for selective protein precipitation. Although these chromatographic systems can provide effective protein purification, they typically have unacceptable economies at large scale (Pearce, 1992). Several attempts have been made to separate the proteins in bovine whey (Kuwata et al., 1985). Forsum (1974) and Mathur and Shahani (1979) attempted the use of sephadex G-75 gel filtration to fractionate whey proteins. Amundson et al. (1982) preferentially precipitated β-lactoglobulin from cheese whey, after concentration by ultrafiltration and demineralization by electrodialysis. Block et al., (1953) reported successful preparation of ferric derivatives of whey proteins. Skudder (1985) showed that sperosil-QMA an anion exchange resin, preferentially absorbed β-lactoglobulin at pH 6.6, with minimal adsorption of the positively charged immunoglobulins, lactoferrin, and lactoperoxidase. Zydney (1998) demonstrated that selective elution of resin-adsorbed whey proteins (β-lactoglobulin, α-lactalbumin, and BSA) can be achieved using hydrochloric acid. Uchida et al. (1996a) reported the purification of lactoferrin and lactoperoxidase by cation exchange chromatography using conventional resins, while Chiu and Etzel (1997) reported the same results using cation exchange membranes. According to Zydney (1998), a variety of affinity ligands have also been examined for whey fractionation, including heparin-sepharose (Blackberg and Hernell, 1980), cibacron blue (Bezwoda and Mansoor, 1986), and specific microclonal antibodies (Kawakami et al., 1987).

Some other studies have accomplished selective precipitation of whey proteins using pH, salts, and temperature treatment (Zydney, 1998). Maillart and Ribadeau-Dumas
(1988) and Mate and Krochta (1994) demonstrated that β-lactoglobulin can be purified by selective precipitation of the other whey proteins at pH 2.0 using 7% sodium chloride. Kaneko et al. (1985) described the use of 7.5mM iron chloride at pH 4.2 and 4°C for the selective precipitation of β-lactoglobulin and BSA, yielding a supernatant concentrated in α-lactalbumin and immunoglobulins. Maubois and Ollivier (1997) reported that immunoglobulins could be selectively precipitated from whey using ammonium or sodium sulfate. However, none of these techniques has been employed at commercial scale, because of the high processing costs and the difficulties associated with the disposal of large quantities of undesirable effluents (Maubois and Ollivier, 1997; Zydney, 1998).

Furthermore, some studies have been based on the use of high temperatures for whey protein fractionation. Pearce (1992) reported the development of a whey purification process based on thermal precipitation, whereby the whey concentrate was heated to 65°C at pH 4.2, which caused α-lactalbumin aggregation and co-precipitation of bovine serum albumins and immunoglobulins (Zydney, 1998). A similar process designed by Maubois et al. (1987) was operated at pH 3.8 and 55°C. A thermal aggregation process for preparing an α-lactalbumin enriched product was described by Uchida et al. (1996b), in which the whey was adjusted to pH 6 and heated to 85°C for 5 min, or to 120°C for 5 sec to aggregate the β-lactoglobulin. Although these whey fractionation processes have generated commercial interest, they have not been widely implemented for large-scale whey protein purification due to their complexity, high cost, low overall yield, poor selectivity, and product degradation due to high temperature treatment (Zydney, 1998).
Membrane processes in whey protein fractionation

Membrane systems are used extensively throughout the dairy industry to control the protein content of milk products. Membrane techniques are especially suitable for the food industry, due to the relatively easy and economic up-scale in comparison to chromatographic techniques (Tolkach and Kulozik, 2005).

Ultrafiltration uses polymeric or ceramic membranes, which are fully retentive to the whey proteins of interest, yielding a retentate stream that can be further processed or utilized. Lactose and mineral content in milk whey can be reduced using a diafiltration process in which deionized water is continually added to the retentate, while lactose and minerals are simultaneously removed in the filtrate (Zydney, 1998). Membrane microfiltration has been examined for the removal of residual lipid from whey prior to ultrafiltration (Lee and Merson, 1976; Merin et al., 1983). Microfiltrations can also be used to remove microorganisms from milk and whey, thus eliminating the need for high temperature treatment (Maubois and Ollivier, 1997). A number of attempts have been made in the last 2 decades to use membrane systems for actual whey protein fractionation. Roger et al. (1984) described a two-stage membrane process for obtaining an α-lactalbumin enriched product from whey. Bottomley (1991) described a similar process using ultrafiltration membranes, whereby the final product had an α-lactalbumin concentration 3 times that of β-lactoglobulin. Scott and Lucas (1989) developed a process for purifying immunoglobulins from whey using ultrafiltration membranes, which allowed permeation of β-lactoglobulin, α-lactalbumin, and BSA, while retaining the immunoglobulins.

It is clear that membranes can be used successfully in several fractionation stages of milk. However, most steps may require further developments for a higher separation
level, purity, or yield (Brans et al., 2004). Reported developments on ultrafiltration are based on two-step fractionation, temperature treatment, surface ionization, acid precipitation, or a combination of some of these processes (Kristansien et al., 1998; Muller et al., 1999; Gesan-Guizion et al., 1999; Mate and Krochta, 1994; Konrad et al., 2000; Maduko et al., 2005c). Using precipitation and ultrafiltration, Gesan-Guizion et al. (1999) reported a purity of 52-83% for α-lactalbumin respectively. Maduko et al. (2005c) described a two-step fractionation process for the selective removal of β-lactoglobulin from goat milk whey. Konrad et al. (2000) compared different purification methods for β-lactoglobulin and obtained purities between 82.5 and 94%. Brans et al. (2004) enhanced ultrafiltration protein selectivity by manipulating the protein properties.

Heat treatment of milk is widely used to modify the properties of milk proteins. The main change that occurs during heat treatment is denaturation of the whey proteins. Singh and Newstead (1992) and Erdem and Yuksel (2005) examined the effect of heat treatment of skim milk on the ultrafiltration process, and reported that heating induces a sieving effect of milk proteins, which contributes to the acceleration of permeate flow during membrane filtration. Several authors have also suggested that cold storage of heat-treated milk may cause rearrangements in the protein system, which positively affects the performance of milk membrane processing (Tanford, 1973; Bonomi et al., 1988; Erdem and Yuksel, 2005).

*Milk fat simulation and modification*

The development of methods to improve the similarity of infant formula lipids to human milk fat (HMF) is of great interest to food processors. Goderis et al. (1987) and Stevenson et al. (1979) suggested that physical properties of milk fat are dependent on
the molecular weight, degree of unsaturation, and positional distribution of fatty acids in the triacylglycerol backbone. Milk fat can be modified for infant feeding by redesigning its physical, chemical, and nutritional properties. The unique fatty acid structure of HMF plays a specific and valuable role in the infants’ growth (Akoh, 2002; Akoh and Xu, 2002). Based on this scientific reasoning, modified fats resembling HMF are being produced by enzymatic interesterification or acidolysis reactions for incorporation in infant formulas (Akoh and Xu, 2002; Sahin et al., 2005).

Lipozyme RM IM enzyme has been reportedly used in interseterification reactions because of its sn-1,3-specificity, that would result in incorporation of unsaturated fatty acids at the sn-1,3 positions of the TAG backbone (Sahin et al., 2005). Innis et al. (1995), Quinlan et al. (1995), and Xu (2000) showed that these unsaturated fatty acids at these positions guarantees maximum fat and calcium absorption in infants and induces efficient use of dietary energy.

**Enzyme-mediated modification of milk fat**

Enzymes are biological catalysts that selectively lower the activation energies of chemical reactions without affecting their chemical equilibrium. Various advantages are derived from the use of enzyme-mediated processes for milk fat modification, which includes action specificity (Macrae, 1983; Gunstone, 1994), waste minimization and mild reaction conditions (Akoh, 2002). According to Desnuelle (1972), lipases are enzymes that specifically hydrolyze fats and oils, acting preferentially at the oil/water interfaces. Use of lipases in milk fat modification gives high selectivity and mimics the natural pathways of metabolic processes. Since a major portion of the energy required by infants is controlled by lipids, the modified fats and oils in infant formulas should have both the correct fatty acid composition as well as the same positional acyl distribution as in HMF.
Modification by enzymatic acidolysis

Enzymatic acidolysis involves the reaction of a fatty acid and triacylglycerol. It is the transfer of an acyl group between an acid and an ester, as an effective means of incorporating free fatty acids into triacylglycerols. Studies by Kalo et al. (1986, 1990) on acidolysis indicate that acyl to fatty acid interchanges occur with interesterification of milk fat in the absence of any solvent and in the presence of a non-specific lipase from *Pseudomonas fluorescens* immobilized on celite. Bornaz et al. (1994) reported the use of an sn-1,3-specific lipase from *Rhizomucor miehei* immobilized on a macroporous anion exchange resin. According to Oba and Witholt (1994), successful acidolysis of milk fat with oleic acid occurred in the presence of a lipase produced by *Rhizopus oryzae* immobilized on controlled pore glass particles. Reyes and Hill (1994) also achieved acidolysis with caprylic acid using a specific lipase from *Pseudomonas cepacia* immobilized unto microporous polypropylene powder. Also interesting is the work of Yang (2003), which reports the production of human milk fat substitutes by lipase-catalyzed acidolysis of lard with soybean fatty acids in a solvent-free stirred reactor. Sahin et al. (2005) also produced structured lipids resembling HMF by enzymatic acidolysis reactions using lipozyme RM IM to catalyze the reaction of fatty acids from hazelnut oil with tripalmitin and stearic acid. By employing the same enzyme, Nielsen et al. (2006) optimized the production of HMF substitutes on a large scale by enzymatic acidolysis of lard and soybean oil using a pilot packed-bed reactor.

Modification by enzymatic interesterification

Interseterification is also known as ester interchange, and is used to exchange and redistribute acyl groups among its triacylglycerol substrates (Akoh, 2002). Hettinga (1996) initially developed an interesterification process using a high-temperature
chemical process and a chemical catalyst (sodium methoxide). Recently, lipases have been used to modify milk fat to exhibit different triacylglycerol stereo-chemical composition, but with the same residue as the starting material (Hettinga, 1996; Kalo et al., 1990). Kalo et al. (1986) modified milk fat using a non-specific lipase from Chromobacterium cylindracea that was immobilized on celite, using hexane as a solvent. Srivastava et al. (2006) utilized Candida rugosa lipase LIP-1 to catalyze transesterification reaction for human fat substitute production. Muhkerjee and Kiewitt (1998) produced structured lipids with similar configurations as human milk fat, for use in infant formula using transesterification process. Many studies have utilized lipozyme RM IM as a biocatalyst for transesterification processes towards human milk fat substitute production (Thompson et al., 1989; Sahin et al., 2005; Sahin et al., 2006). Recently, Sahin et al. (2006) reported the successful production of a structured lipid containing the long-chain polyunsaturated fatty acids- eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). According to these authors, the structured lipid was aimed to deliver both the health benefits associated with omega-3 fatty acids, and the absorption characteristics and fatty acid composition similar to those of human milk fat.

LITERATURE CITED


composition of erythrocytes in full term infants fed breast milk, commercial
formula or evaporated milk. Journal of Developmental and Behavioural

508-511.

Davidson, G.P. and Townley, R.R.W. 1977. Structural and Functional abnormalities of
the small intestine due to nutritional folic acid in infancy. J. Pediatr. 90: 590-596.

Davis, T.A., Nguyen, H.V., Garcia-Bravo, R., Floroto, M.L., Jackson, E.M. Lewis, D.S.,


Debski, B., Picciano, M.F. and Milner, J.A. 1987. Selenium content and distribution of


composition during late lactation (7-20 months). Journal of Pediatric
Gastroenterology and Nutrition 3: 713-720.

Conjugated linoleic acid content of milk and cheese from cows fed extruded oil
seeds. J. Dairy Sci. 82: 412-419.


coordinated international expert group. J. Pediatric Gastroenterology and Nutrition. 41: 584-549.


Le Jaouen, J.C. 1987. The making of farmstead goat cheeses. Cheesemakers J., P.O.Box 85, Ashfield M.A.


CHAPTER 3

COMPARISON OF FATTY ACID AND STEROL COMPOSITION OF MODIFIED
GOAT MILK PRODUCTS FOR INFANT FORMULAE

11/21/2006
ABSTRACT

Goat milk was modified with vegetable oils to simulate lipid content of human milk for infant feeding. Five different blends of vegetable oils were prepared and added separately to skim goat milk (SGM) at a rate of 4.4 g oil per 100 mL milk. The unmodified goat milk (GM), SGM, and the 5 formulated milks were analyzed for fatty acid profiles and sterol contents by gas chromatography. The formula modified with 2.5/1.1/0.8 (v/v/v) coconut, safflower, and soybean oils had the closest similarity in fatty acid profile to human milk. The total sterol contents of GM, SGM, HM and the formulated milk groups were 11.6, 10.4, 19.1, 21.6, 26.6, 26.9, 21.2, and 21.3 mg 100 mL⁻¹ respectively. The phytosterol contents of the formulated milks were closer to that of human milk than the GM. The formulated milks also contained appreciable quantities of stigmasterol, campesterol and beta-sitosterol, as in human milk.

KEYWORDS: Fatty acid, sterol, goat milk, vegetable oil blends, formulated milk, infant food.

INTRODUCTION

The demand for alternatives to human milk has been never-ending due mainly to the inability of some mothers to breast-feed, which may cause serious problems for the survival of the newborn infants. However, alternative foods such as infant formulas must meet the nutritional requirements of the growing infants as closely as possible (Fomon, 1993, Forsyth, 1998).
Formulated goat milk has been recommended as a good substitute for cow milk and is becoming increasingly popular for use as food for infants that are allergic to cow milk from the first year of life (Taitz & Armitage, 1984; Park, 1994). Goat milk is reportedly less allergenic and more digestible than cow milk (Birkbeck, 1978; Kirke, 1979; Taitz & Armitage, 1984; Park, 1994; Park & Haenlein, 2006), and virtually all infants who are sensitive to cow milk can tolerate goat milk (Bahner & Heiner, 1980, Coveney & Darton-Hill, 1985, Park, 1994, Park & Haenlein, 2006). Although goat milk production is approximately 2 % of the total world milk production, its economic importance is substantial in most developing and some developed countries, where cow milk is not available and climatic conditions are not favorable for cattle raising (Park & Chukwu, 1988; Park, 1991; Crepaldi, Corti, & Cocogna, 1999).

Fat is the major source of energy in human milk and contributes about 50 % of the total energy for infants (Jensen, 1989a, 1989b). Likewise, the lipid component of formulae for infant feeding acts as both the major source of energy and the vehicle for the provision of fat-soluble vitamins and essential fatty acids in infants’ diet (Hamosh, Bitman, Wood, Hamosh & Melita, 1985). Goat milk contains 3.8 g fat per 100 mL, the Food and Drug Administration (1985) previously recommended that the total lipid content of infant formulas be in the range of 3.3-6.5 g per 100 mL. However, suggestions regarding the unsuitable energy content of low-fat formulas resulted in the recent stipulation that infant formulas should have about 4.4 g fat per 100 mL, which is similar to that of human milk values (ESPGHAN Committee on Nutrition, 1991).

More than 200 fatty acids have been identified in human milk, with oleic (C18:1), palmitic (C16:0), lauric (C12:0), linoleic (C18:2); myristic (C14:0), stearic (C18:0), and capric acids (C10:0) comprising 90 % of total fat (vanBeusekom et al., 1993). Palmitic
acid is quantitatively the predominant saturated fatty acid in human milk triacylglycerols (TAGs) and accounts for approximately 23 % of total milk fatty acids (Innis, 1992). Oleic acid represents about 90 % of the monounsaturated fatty acids and about 38 % of total fatty acids present in human milk. The fat composition of goat milk on the other hand, is typical of ruminant milk in general. It is a relatively poor source of essential fatty acids (Parkash & Jenness, 1968, Le Jaouen, 1981), and contains approximately two-thirds by weight of saturated fatty acids. It would be therefore necessary to develop products that are more nutritionally desirable and homologous to human milk fat for infant feeding through adjustment of the composition of goat milk fat to more closely resemble that of human milk. Furthermore, the human infant needs cholesterol for growth and development, including that of cognitive, visual, and neurological functions (Fomon, 1993). The cholesterol content of a typical commercially-available infant formula is usually less than one-third of that found in human milk (Forsyth, 1998).

No studies have been published on the distribution of fatty acids and the sterol content of goat-milk-based infant formulae containing blends of vegetable oils as the predominant lipid source. Coconut oil was used in this study as the source of medium- and long-chain saturated fatty acids, while safflower and soybean oils were used as sources of long-chain monounsaturated and polyunsaturated fatty acids. For the successful production of infant formulae for human milk replacement, a blend of vegetable oils is required to simulate human milk fat profile as closely as possible. Therefore, the purposes of this study were: (1) to develop infant milk fat analogues using different blends of coconut, safflower and soybean oils, and (2) to compare the fatty acid profiles and cholesterol content of goat milk infant formula analogues to those of human milk.
MATERIALS AND METHODS

Preparation of goat milk

Fresh goat milk (GM) was obtained from the Georgia Small Ruminant Research and Extension Center at Fort Valley State University Fort Valley (GA, USA). GM was pasteurized at 63 °C for 30 min and the fat was separated from the milk using a milk cream separator (Armfield FT15, Ringwood, Hampshire, England) at a low speed of 480 x g to give skim goat milk (SGM) having 0.17 g 100 g⁻¹ residual fat.

Preparation of vegetable oil blends

Coconut, Safflower, and Soybean oils were purchased from local retail outlets (Athens, GA, USA). Five different blends of coconut, safflower, and soybean oils were prepared using the following combination ratios: B1 (2.5:1.1:0.8), B2 (0.8:2.5:1.1), B3 (1.1:0.8:2.5), B4 (2.0:1.4:1.1) and B5 (1.5:1.4:1.5).

Preparation of milk formulae

The SGM was divided into 5 equal portions. One portion of the SGM was mixed separately with each of the 5 different vegetable oil blends at a rate of 4.4 g oil per 100 g milk to make 5 corresponding formulated milk blends: F1, F2, F3, F4, and F5. The milk blends were homogenized using a laboratory-scale homogenizer (APV Gaulin 15MR-8TBA 78911183 Lake Mills, WI, USA), after which GM and the milk blends were lyophilized at −40 °C and a pressure of 133 ×10⁻⁴ kPa using a freeze-drier (Virtis, Gardiner, NY, USA). The formulation of the 5 vegetable oil blends and 5 goat milk blends were prepared in 2 replicates, and triplicate samples were taken from each replicate for evaluation of fatty acids composition and sterol concentration.
Preparation of chemicals

Methanol, chloroform and other organic solvents and chemicals were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) or Fisher Scientific (Narcoess, GA, USA). Lipid standards, including heptadecanoic acid and methyl esters of caprylic, capric, lauric, myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, arachidonic, and behenic acids, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were ACS and reagent grades.

For cholesterol analysis, the following reagents and sterol standards were prepared: saturated potassium hydroxide (KOH) in water for saponification, 30 g L⁻¹ pyrogallol in ethanol as antioxidant during saponification, butylated hydroxytoluene (5 mg mL⁻¹) as antioxidant in both the internal, cholesterol, stigmasterol, beta-sitosterol, and campesterol standards, 5-β cholestane in toluene (100 μg 100mL⁻¹) as internal standard, cholesterol standard in toluene (1 mg mL⁻¹) and sterol standards in hexane (0.25 mg mL⁻¹).

Extraction of lipids

Lipid from GM was extracted by the procedure described in Folch, Lees and Stanley (1957). One gram of the lyophilized sample was homogenized with 12 mL of pre-chilled chloroform-methanol (2:1 v/v) containing 0.005 % butylated hydroxytoluene (BHT). The sample tubes were centrifuged at 800 x g for 5 min to separate insoluble particles, and then the solvent layer was passed through a sodium sulfate column. The solvent was evaporated in a 50 mL evaporation flask using a rotary evaporator (Labconco KRvr TD 65/45, Kansas City, MO, USA) at 60 °C. The dried fat extract was reconstituted with 2 mL chloroform-methanol and flushed with nitrogen.
Preparation of fatty acid methyl esters (FAME)

A 200 μL sample each of coconut, safflower, and soybean oils, and of the five vegetable oil blends was pipetted into a screw-cap (25 × 200 mm) reaction tube. Heptadecanoic acid (20 μL) was added to each tube as an internal standard. Excess solvent was evaporated from each tube, after which 3 mL of 6 % hydrochloric acid in methanol was added and the mixture was methylated by incubation at 75 °C for 2 h in a pre-heated oven. Upon methylation, the mixture was extracted twice with 2 mL hexane and 1 mL of 0.1 M potassium chloride and centrifuged at 400 x g for 3 min. The upper (hexane) layer was decanted and both extracts were combined for each sample, and then passed through pasteur pipettes packed with sodium sulfate to remove moisture. The excess solvent was evaporated under nitrogen (N-EVAP Organomation, Model No. 111, Berlin, MA, USA) until about 1 mL was obtained.

Gas chromatographic (GC) analysis of FAMEs

Fatty acid profiles were quantified using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID). Helium was the carrier gas and the gas flow rate was 1.7 mL min⁻¹. The oven temperature was initially held at 80 °C for 3 min and then programmed to 215 °C at 10 °C min⁻¹ increase and held isothermally for 20 min. The column used was a fused silica Heliflex capillary column (Alltech-AT-225: 30 mm × 0.25 mm × 0.25 μm film thickness; Alltech Associates Inc., Deerfield, IL, USA). Concentrations of FAMEs (mol %) were analyzed and integrated using an integrator (Model no. G2070AA, Agilent Technologies Inc., Palo Alto, CA, USA) with reference to C17:0 as the internal standard.
Determination of cholesterol

Cholesterol concentrations of GM, SGM, and formulated milk samples were determined using the method of Jekel, Vaessen and Schothorst (1998).

Saponification and extraction

Saponification of lipid samples was performed according to the method of (Fatouh, Singh, Koehler, Mahran, & Metwally, 2005). One gram of lipid extract was weighed into screw-cap test tubes (25×200 mm). A 10 mg internal cholesterol standard (5-β cholestane) was added to each tube, and the tubes were flushed with nitrogen for 2 min to prevent oxidation. Then 8 mL pyrogallol solution and 0.5 mL KOH solution were added to the tubes. The tube contents were thoroughly mixed by vortexing (5 min) and then placed in a water bath at 80 °C for 30 min. After cooling to room temperature, sterols were extracted by adding 20 mL hexane and 12 mL deionized water into each tube, and vortexed again for 5 min. The tubes were left for 15 min to allow separation of hexane and aqueous layers. A 10 mL aliquot from the top hexane layer was taken from each tube and the vial content was evaporated to dryness using nitrogen stream. One mL hexane was added to each vial and the resultant hexane solution was used in gas chromatography (GC).

GC analysis of cholesterol

One-µl aliquot of hexane extract was injected into a GC (HP5980 series II GC; Hewlett-Packard, San Fernando, CA, USA) equipped with a flame ionization detector (FID) and on-column injector. The column used was a fused silica capillary column (SAC-5; 30 mm × 0.20 mm i.d. × 0.20 µm film thickness; Supelco, Bellefonte, PA, USA). Nitrogen was the carrier gas at a flow rate of 20 mL min⁻¹ and hydrogen and air were supplied at a flow rate of 33 and 400 mL min⁻¹, respectively. The oven temperature was programmed at 20
°C min\(^{-1}\) from 155 to 275 °C and held for 30 min isothermally at 275 °C. The injector and detector temperatures were 270 °C and 300 °C, respectively. The oven temperature was programmed at 20 °C min\(^{-1}\) from 155 to 275 °C and held for 30 min isothermally at 275 °C.

*Statistical analysis*

All experimental data collected from the 5 formulated milks were statistically analyzed by analysis of variance, and least squares mean comparison among different treatments with reference to human milk composition, using the general linear model (GLM) of SAS program (SAS, Cary, NC, USA).

**RESULTS AND DISCUSSION**

*Fatty acid profile*

The fatty acid profile of the vegetable oils (Table 3.1) showed appreciable quantities of the short- and medium-chained saturated fatty acids (coconut oil), long-chain monounsaturated fatty acids (safflower oil), and long-chain polyunsaturated fatty acids (soybean oil). Lauric acid contributes about 50 % of the total fatty acids in coconut oil, which also contributes about half of the saturated fatty acids in this oil. Oleic acid contributes about 74 % of the total fatty acids and 84 % of the unsaturated fatty acids in safflower oil, while the soybean oil contained about 21 % oleic acid and approximately 50 % linoleic acid. About 16 % of the unsaturated fatty acids of safflower oil consist of linoleic acid, where this fatty acid also makes up about 64 % of the unsaturated fatty acids in soybean oil.

GM had appreciable quantities of short- and medium-chain saturated fatty acids, and unsaturated fatty acids composed mainly of palmitoleic, oleic, and linoleic acids.
(Table 3.2). This is quite different from the fatty acid profile of the formulated goat milk blends, which consisted mainly of the medium- and long-chain fatty acids, with unsaturated fatty acids ranging from oleic (C18:1) to arachidonic acid (C20:4), though C20:1 and C20:4 were present in trace amounts. The formulated milks contained higher lauric acid values than the Codex limit of 15 % of total fat. The fatty acid profiles of the formulated milks also appeared different from that of the cow milk-based infant formula (CIF), with the formulated milks having lower contents of C4:0, C6:0 and C16:0, and significantly (p < 0.05) higher contents of the medium chain saturated fatty acids (C8:0 to C14:0). The C18:0 contents of F1 and F4 were significantly (p < 0.05) higher than that of CIF, while the long chain polyunsaturated fatty acids content of CIF, F1, F2, and F3 appeared comparable to that of HM.

The highest percentage of fatty acid in HM is oleic acid (38 %), followed by palmitic acid (23 %), linoleic acid (9 %), and myristic acid (8 %). The capric, myristic stearic, palmitic, and oleic acids in HM accounted for approximately 78 % of total fatty acids, with oleic and palmitic acids contributing more than 75 % to the total (Table 3.2). However this is not the case for GM, which consists of 30.8 % palmitic acid, 18.23 % oleic acid, 9.8 % capric acid, and 9.6 % myristic acid. These fatty acids, in addition to stearic acid, comprised about 75 % of total fatty acids in goat milk, with oleic and palmitic acids contributing less than 65 % of the total (Alonso, Fontecha, Lozada, Fraga, & Juarez 1999). Since human milk fat is the major source of energy, fat soluble vitamins and essential fatty acids for the human infant, an alternative source of fat for infant feeding should therefore simulate the fat composition of human milk as closely as possible. This would ensure the same growth and development outcome in infants fed alternative milks as in breast-fed infants.
On the other hand, the formulated milk groups contained closer levels of oleic acid to HM than GM contained. The formulated milk groups had the highest oleic acid content among all fatty acids, with F2 having the highest (Table 3.2). However, the levels of the major saturated fatty acids such as palmitic (C16:0) and stearic (C18:0) acids in the formulated milks were significantly (P < 0.05) lower than that of GM, while the opposite trend occurred for lauric acid (C12:0) content (Table 3.2).

The linoleic acid content of F1, F2, and F3 formulated milks were also very similar to that of HM, with F1 (9.2 %) giving the closest value. These values appear to meet the lower limit specified by the Codex regulations for this fatty acid. The lauric, myristic, palmitic, oleic, and linoleic acid contents of HM accounted for about 85 % of the total fatty acids, with oleic acid contributing nearly 50 % of this total. The percent contribution of these fatty acids to F1, F2, F3, F4, and F5 groups were 82.6, 88.1, 84.9, 85.0, and 81.9 % respectively, while oleic acid contents alone for the corresponding groups were approximately 42.5, 51, 44.7, 50.6, and 48.9 % of the totals (Table 3.2).

The percentages of saturated, monounsaturated, and polyunsaturated fatty acids of the F2 milk blend were 43.1, 45.0, and 10.6 % (Figure 3.1). In this attribute, the F2 formulation appeared to have a close similarity to those of HM (47, 42, and 10.8 %), followed by those of F1, with 54.7, 35.1, and 10.2 %, respectively. Those close similarities of both F2 and F1 to human milk are attributable to lower ratios of coconut oil content which consists mostly of medium-chain saturated fatty acids, and higher ratio of safflower and soybean oil content, which contributes a high percentage of long-chain unsaturated fatty acids. Safflower and soybean oils are two sources of polyunsaturated fatty acids (Packard, 1982) that, together with coconut oil, produce an acceptable combination of fat that can be employed in infant food formulations (Maduko, Peck,
Toledo & Park, 2005). Although the percent monounsaturated fatty acids of the formulated milks appeared comparable to that of CIF, these formulated milks had higher percent saturated fatty acids levels and lower percent polyunsaturated fatty acids than CIF (Figure 3.1).

The polyunsaturated-to-saturated fatty acid ratio (P/S) in HM is about 0.23 (Jensen, 1989a), which is closely simulated by the P/S ratio of F1 (0.19), F2 (0.25), and F3 (0.21). HM and the formulated milks showed lower P/S ratios than CIF (Figure 3.2). Koletzko and Bremer (1989) observed that most manufacturers try to produce formulas with P/S ratios ranging from 0.20-0.50. The low P/S values of GM, F4, and F5 milk samples might have resulted from the low unsaturated contents of fatty acid in these groups.

Cholesterol and sterol contents

The total cholesterol in fluid GM is about 10.5-12.8 mg 100 g⁻¹, which is about 14 % less than the amount present in human milk (Table 3.3). Although Hachey, Pond, and Wong (1996) reported the total daily cholesterol required by infants for growth and metabolism to be in the range 1.5-2.0 mg 100 g⁻¹ milk, human milk contains about 14 mg cholesterol per 100 g of fluid breast milk, which is the highest among all treatment groups (Fig 3.2).

The cholesterol content in human milk accounts for about 71 % of the total sterols, while in goat milk cholesterol is estimated at about 98 % of total sterols (Contarini, Povolo, Bonfitto, & Berardi, 2002), which is in agreement with the results of the present study (Table 3.3). The formulated milk blends had cholesterol quantities that is comparable to that of goat milk (Fig 3.2), and significantly (p < 0.05) lower and higher than that of CIF and HM respectively. Although F1 appears to have the closest content to
human milk, with a cholesterol level of 9.8 mg 100 g⁻¹, there was no significant difference (p < 0.05) in cholesterol content between the 5 different formulated goat milk blends (Table 3.3).

The phytosterols occurred at very low levels in GM sample (1.6 % of total sterols), whereas higher levels, about 34 %-50.5 % of total sterols, were contained in the formulated milk blends (Figure 3.2). There appeared to be lower levels of phytosterols and total sterols in HM and the formulated milks than in CIF (Figure 3.2). The F1 had the lowest occurrence of phytosterols, while the F2 and F3 milk blends had the highest. However, there appear to be no significant (p > 0.05) difference between the phytosterol content of F2 and F3 milk blends (Table 3.3). These results showed slightly higher total sterol levels in formulated milks than in HM, though there appears to be no significant (p > 0.05) difference between HM, F1, F4, and F5 values of this index. Furthermore, GM showed no detectable quantities of stigmasterol and campesterol, while both were present at appreciable quantities in HM and the milk blend samples. Other sterols, such as beta-sitosterol, are also present in human breast milk. Mellies, Glueck, Sweeney, Fallat, Tsang, and Ishikawa (1976) reported phytosterol content as high as 5 % of total milk fat (220 mg 100 g⁻¹ of milk fat) in human milk. This is usually observed when maternal diets are rich in phytosterols, resulting in approximately 50 % of the total sterols being present as phytosterols in human milk (Mellies et al., 1976). According to Mellies et al., 1976, the presence of high levels of phytosterols in human breast milk also can be explained by the inclusion of high amounts of vegetables or vegetarian foods in the maternal diet.
CONCLUSIONS

This study has shown that vegetable oil blends of coconut, safflower, and soybean oils can be used for milk fat formulations for infant food, which appears to be important since unmodified goat milk is not suitable for infants in the first year of life. Several of the formulated goat milk samples appeared to meet the Codex regulations for fat content, although they contained higher lauric acid (C12:0) content than the 15 % of total fat content specified by the current EU regulations. The milk formulated with a blend of 2.5 g coconut oil, 1.1 g safflower oil, and 0.8 g soybean oil (F1), appeared to have the closest similarity in fatty acid profile to that of human milk, in comparison with those of whole goat milk and the other formulated milk blends, though there are significant (p<0.05) differences between the medium chain saturated fatty acids of this formula (F1) and human milk. The fatty acid profile of the modified goat milk was comparable to that of human milk, with similar levels of oleic (35.1 %), linoleic (9.2 %), and linolenic acids (1 %). Unlike whole goat milk, this formulated milk also did not contain C4:0 and C6:0 fatty acids, both of which are absent in human milk. The phytosterol and total sterol contents of the modified milk, relative to whole goat milk, were closer to that of human milk, whereby the formulated fat may be suitable for the development of infant milk fat and infant foods.

ACKNOWLEDGEMENT

This research was supported by the grants of SARE (Southern Region Research and Education)/USDA No. RD309-032/1575557 and USDA 1890 Capacity Building grant No. 2001-38814-11388. The authors thank Dr. Philip Koehler for the use of his
laboratory, Anne Morrison and Brenda Jennings for their laboratory assistance, and Mr. Schauston Miller for collection of goat milk samples.

LITERATURE CITED


Table 3.1: Fatty acid profile of vegetable oils (% w/w)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Coconut oil</th>
<th>Safflower oil</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>Caprylic</td>
<td>15.62±0.09</td>
<td>ND</td>
</tr>
<tr>
<td>C10:0</td>
<td>Capric</td>
<td>10.27±0.03</td>
<td>ND</td>
</tr>
<tr>
<td>C12:0</td>
<td>Lauric</td>
<td>50.75±0.12</td>
<td>ND</td>
</tr>
<tr>
<td>C14:0</td>
<td>Myristic</td>
<td>12.97±0.08</td>
<td>ND</td>
</tr>
<tr>
<td>C16:0</td>
<td>Palmitic</td>
<td>3.27±0.04</td>
<td>9.22±0.15</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic</td>
<td>5.44±0.01</td>
<td>1.96±0.05</td>
</tr>
<tr>
<td>C18:1</td>
<td>Oleic</td>
<td>1.29±0.08</td>
<td>74.01±0.20</td>
</tr>
<tr>
<td>C18:2</td>
<td>Linoleic</td>
<td>0.34±0.01</td>
<td>14.82±0.11</td>
</tr>
<tr>
<td>C18:3</td>
<td>Linolenic</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C20:1</td>
<td>Gadoleic</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Tr: Trace (< 0.01)

ND: Not detected
Table 3.2: Comparison of fatty acid profiles of goat milk and formulated milks with that of human milk.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>GM</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>CIF</th>
<th>HM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>Butyric</td>
<td>2.2±0.0*a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1b</td>
</tr>
<tr>
<td>C6:0</td>
<td>Caproic</td>
<td>2.4±0.1*a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1b</td>
</tr>
<tr>
<td>C8:0</td>
<td>Caprylic</td>
<td>2.7±0.1*c</td>
<td>4.9±0.1*ab</td>
<td>2.2±0.2*ab</td>
<td>3.7±0.2*ab</td>
<td>5.6±1.4*</td>
<td>5.4±0.36*</td>
<td>0.9d</td>
</tr>
<tr>
<td>C10:0</td>
<td>Capric</td>
<td>9.9±0.2*a</td>
<td>6.4±0.1*e</td>
<td>7.1±0.1*cd</td>
<td>8±0.2*cd</td>
<td>8.7±0.8*bc</td>
<td>9.5±0.01*bc</td>
<td>1.0f</td>
</tr>
<tr>
<td>C12:0</td>
<td>Lauric</td>
<td>4.9±0.1d</td>
<td>26.6±0.7*a</td>
<td>18.2±0.5*</td>
<td>22±0.9*</td>
<td>26.1±4.7*</td>
<td>23.6±0.08*</td>
<td>12.2*</td>
</tr>
<tr>
<td>C14:0</td>
<td>Myristic</td>
<td>9.7±0.0*bc</td>
<td>12.5±0.0*c</td>
<td>8.8±0.1*</td>
<td>9.3±0.2*bc</td>
<td>9.6±1.2*bc</td>
<td>10±0.05*</td>
<td>4.8*</td>
</tr>
<tr>
<td>C16:0</td>
<td>Palmitic</td>
<td>30±0.2*a</td>
<td>9.2±0.2 d</td>
<td>6.2±0.1*</td>
<td>6.4±0.3*</td>
<td>3.5±0.5*</td>
<td>4.20±0.02*</td>
<td>18.2*</td>
</tr>
<tr>
<td>C16:1</td>
<td>Palmitoleic</td>
<td>1.6±0.1*b</td>
<td>0.1±0.0*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.1*</td>
<td>3*</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic</td>
<td>8.0±0.5*a</td>
<td>5.1±0.0*e</td>
<td>1.0±0.1*</td>
<td>2.0±0.0*</td>
<td>4.7±0.1*</td>
<td>2.61±0.01*</td>
<td>3.2*</td>
</tr>
<tr>
<td>C18:1</td>
<td>Oleic</td>
<td>18.2±0.4*</td>
<td>35.1±0.3*</td>
<td>45±0.5*</td>
<td>38±0.4*</td>
<td>43±0.9*</td>
<td>39.9±0.2*</td>
<td>41.5*</td>
</tr>
<tr>
<td>C18:2</td>
<td>Linoleic</td>
<td>3.3±0.1*e</td>
<td>9.2±0.2*</td>
<td>9.9±0.2*</td>
<td>9.5±0.3*bc</td>
<td>2.8±0.2*</td>
<td>4.2±0.14*</td>
<td>13.4*</td>
</tr>
<tr>
<td>C18:3</td>
<td>Linolenic</td>
<td>0.4±0.0*df</td>
<td>1±0.0*</td>
<td>1.7±0.0*</td>
<td>1.2±0.0*</td>
<td>0.2±0.0*</td>
<td>0.45±0.02*</td>
<td>2.8*</td>
</tr>
<tr>
<td>C20:1</td>
<td>Gadoleic</td>
<td>-</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>0.5*</td>
</tr>
<tr>
<td>C20:4</td>
<td>Arachidonic</td>
<td>-</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>-</td>
</tr>
<tr>
<td>C22:1</td>
<td>Erucic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Tr</td>
</tr>
</tbody>
</table>

ND= Not Detected; Tr = Trace (<0.05); GM: Goat milk; F1: Milk formulated with a blend of 2.5 g coconut oil, 1.1 g safflower oil and 0.8 g soybean oil; F2: Milk formulated with a blend of 0.8 g coconut oil, 2.5 g safflower oil and 1.1 g soybean oil; F3: Milk formulated with a blend of 1.1 g coconut oil, 0.8 g safflower oil and 2.5 g soybean oil; F4: Milk formulated with a blend of 2.0 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; F5: Milk formulated with a blend of 1.5 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; HM: Human milk; * Data obtained from USDA (1976); CIF: Cow milk-based infant formula; **Data obtained from Lopez-Lopez et al. (2002); Data with the same letter within a row are not significantly different (p < 0.05).
Table 3.3: Cholesterol, campesterol, stigmasterol and beta-sitosterol contents (mg/100g) of milk samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cholesterol</th>
<th>Campesterol</th>
<th>Stigmasterol</th>
<th>Beta-sitosterol</th>
<th>Other sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM</td>
<td>11.2±1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>SGM</td>
<td>10.4±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F1</td>
<td>9.8±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5±0.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.5±1.1&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F2</td>
<td>9.7±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>F3</td>
<td>9.8±1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F4</td>
<td>9.9±2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.7±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5±0.1&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F5</td>
<td>9.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.8±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7±1.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIF**</td>
<td>4.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>14.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>HM*</td>
<td>14.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9&lt;sup&gt;d&lt;/sup&gt;***</td>
<td>0.97&lt;sup&gt;c&lt;/sup&gt;***</td>
<td>1.7&lt;sup&gt;cd&lt;/sup&gt;***</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;***</td>
</tr>
</tbody>
</table>

GM: Goat milk; SGM: Skim goat milk; F1: Milk formulated with a blend of 2.5 g coconut oil, 1.1 g safflower oil and 0.8 g soybean oil; F2: Milk formulated with a blend of 0.8 g coconut oil, 2.5 g safflower oil and 1.1 g soybean oil; F3: Milk formulated with a blend of 1.1 g coconut oil, 0.8 g safflower oil and 2.5 g soybean oil; F4: Milk formulated with a blend of 2.0 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; F5: Milk formulated with a blend of 1.5 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; HM: Human milk; *Data obtained from Jensen (1989); CIF: Cow milk-based infant formula; **Data obtained from Huisman et al., 1996; *** Calculated from total of 'phytosterols’ in human milk; Data with the same letter within a column are not significantly different (p < 0.05).
Figure 3.1: Total percentage (w/w) saturated, monounsaturated, polyunsaturated and P/S ratio of different milk treatment groups. GM: Goat milk; SGM: Skim goat milk; F1: Milk formulated with a blend of 2.5 g coconut oil, 1.1 g safflower oil and 0.8 g soybean oil; F2: Milk formulated with a blend of 0.8 g coconut oil, 2.5 g safflower oil and 1.1 g soybean oil; F3: Milk formulated with a blend of 1.1 g coconut oil, 0.8 g safflower oil and 2.5 g soybean oil; F4: Milk formulated with a blend of 2.0 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; F5: Milk formulated with a blend of 1.5 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; CIF: Cow milk-based infant formula (Lopez-Lopez et al., 2002); HM: Human milk (USDA, 1976). Sample number (n) = 6.
Figure 3.2: Percentage (w/w) concentrations of total sterols, cholesterol and phytosterols in goat, formulated and human milk fats. GM: Goat milk; SGM: Skim goat milk; F1: Milk formulated with a blend of 2.5 g coconut oil, 1.1 g safflower oil and 0.8 g soybean oil; F2: Milk formulated with a blend of 0.8 g coconut oil, 2.5 g safflower oil and 1.1 g soybean oil; F3: Milk formulated with a blend of 1.1 g coconut oil, 0.8 g safflower oil and 2.5 g soybean oil; F4: Milk formulated with a blend of 2.0 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; F5: Milk formulated with a blend of 1.5 g coconut oil, 1.4 g safflower oil and 1.5 g soybean oil; CIF: Cow milk-based infant formula (Huisman, 1996); HM: Human milk (Jensen, 1989). Sample number (n) = 6.
CHAPTER 4

ENZYMATIC INTERESTERIFICATION OF TRIPALMITIN WITH VEGETABLE OIL BLENDS FOR FORMULATION OF CAPRINE MILK INFANT FORMULA ANALOGS\textsuperscript{1}


Reprinted here with permission of the publisher.
ABSTRACT

The structure of triacylglycerols in vegetable oil blends was enzymatically modified, and the blends were incorporated into skimmed caprine milk to produce goat milk based infant formula analogs, homologous to human milk fat. A modified lipid, containing palmitic, oleic and linoleic acids, resembling the composition of human milk fat, was synthesized by enzymatic interesterification reactions between tripalmitin and a vegetable oil blend containing 2.5:1.1:0.8 ratio of coconut, safflower and soybean oils. Commercial sn-1,3-specific lipase obtained from Rhizomucor miehei, Lipozyme RM IM, was used as the biocatalyst. The effects of substrate molar ratio and reaction time on the incorporation of palmitic, oleic and linoleic acids at the sn-2 position of the triacylglycerols were investigated. Fatty acid composition and sn-2 position of the experimental formulas were analyzed using gas chromatography. Results showed that the highest palmitic acid incorporation was obtained at 12 hr incubation at 55 °C with a substrate molar ratio of 1:0.4 of tripalmitin to vegetable oil blends. However, the modified milk interesterified for 12 hr at 1:1 molar ratio had a better resemblance to human milk in compared with the other formulas. The level of oleic acid incorporation at the sn-2 position increased with the molar ratio of tripalmitin to vegetable oil blend. It was concluded that unlike the original goat milk and other formulas, the formulated caprine milk with a molar ratio of 1:1 and 12 h incubation was similar to the fatty acid composition of human milk.

KEY WORDS: Enzymatic interesterification, tripalmitin, vegetable oil blend, caprine milk, infant formula Analog.

INTRODUCTION

Human milk is considered as nature’s best infant food from nutritional, immunological and food safety points of view (Megraud et al., 1990). However, time
constraints and urbanization may cause the early termination of breast-feeding. Furthermore, some infants are not breast-fed because of a short supply of human milk, insufficient nutrition and health conditions of the nursing mother, the death of the mother during or after childbirth, and the necessity of some mothers having to work.

Therefore, the need exists to provide an alternative means of feeding for those infants who cannot be breast-fed. As the demand for an alternative to breast milk continues, a substitute of human milk should as closely as possible meet the nutritional requirements of the rapidly growing infant (Forsyth, 1998). The ultimate goal of designing an infant formula is to achieve the same outcomes as seen in breast-fed infants (British Department of Health, 1996).

Human milk consists of 4.4 % total lipids, and human milk fat (HMF) consists mostly of long-chain fatty acids such as palmitic, oleic, linoleic, and stearic acids. Unlike the palmitic acid in vegetable oils and ruminant milk fats, the palmitic acid in HMF constitutes the highest proportion (53-70 %) of saturated fatty acids at the sn-2 position of the triacylglycerol (TAG) backbone, and unsaturated fatty acids are at the sn-1 and sn-3 positions (Jensen, 1989; Innis et al., 1995; Xu, 2000). The location of palmitic acid at the sn-2 position of TAGs in HMF increases the absorption of palmitic and stearic acids in the lumen of infants and decreases the loss of calcium in their feces (Quinlan et al., 1995; Kennedy et al., 1999). This is due to the preservation of the sn-2 positional palmitic acid during digestion, absorption, and biosynthesis of TAGs in the intestinal wall.

Milk fat can be modified for infant feeding by redesigning its physical, chemical and nutritional properties. Modified lipids resembling the TAGs of human milk can be produced by interesterification, using sn-1,3-specific lipase as the biocatalyst (Sahin et al., 2005a,b), and use of this lipase gives high selectivity and mimics the natural
pathways of metabolic processes. Since a major portion of the energy required by infants is provided by lipids, the modified fats and oils in infant formulas should have both the correct fatty acid composition and the same positional acyl distribution as in HMF.

Caprine milk has been recommended as a potential alternative to human milk for infant feeding (Birkbeck, 1978; Kirke, 1979; Taitz and Armitage, 1984) attributable to its less allergenic and more digestible properties compared to bovine milk (Park, 1991; Park, 1994; Park and Haenlein, 2006). A significant amount of caprine milk produced in the world is consumed by infants and patients who suffer from allergy to cow’s milk (Park, 1994; Park and Haenlein, 2006). Infants’ diet in Australia, Italy, South Wales and North Africa consists chiefly of caprine milk (Brande, 1972).

However, the positions of fatty acids (especially at the sn-2) in TAGs of goat milk fat are different from those of HMF. It is beneficial to modify the TAGs of goat milk to become homologous to HMF so that the absorption milk fats in caprine milk infant formulas would be similar to that of breast milk. Therefore, the objective of this study was to produce caprine milk infant formula analogs containing similar fatty acid profile and TAG composition similar to human milk fat for infant feeding.

MATERIALS AND METHODS

Preparation of Materials and Experimental Procedures

Tripalmitin (glycerol tripalmitate, minimum purity of 85 %) and porcine pancreatic lipase (type II, crude) were purchased from Sigma Chemical Co. (St. Louis, MO). Immobilized 1,3-specific lipase, Lipozyme RM IM, was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Organic solvents and thin-layer chromatography (TLC) plates were purchased from J. T. Baker Chemical Co. (Phillisburg, NJ) and Fisher
Scientific (Fair Lawn, NJ), respectively. All solvents and reagents used for sample analyses were chromatographic or analytical grade.

Preparation of goat milk and vegetable oil blends

Fresh goat milk was obtained from the bulk tank milk of mid-lactation Saanen, Nubian, and Alpine dairy goat breeds at the Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, GA. The average daily milk yield was 1.1 L/head and the average somatic cell count of the milk was 550,000/ml. The goats were fed bermudagrass hay ad libitum, and 1 lb of concentrate feed twice daily. The concentrate contained 5.0% crude fat and 2.72% digestible energy, which ensured 17% of fiber in the total diet.

On the basis of our preliminary studies (Maduko et al., 2005, 2006) and the results of Packard (1982), a vegetable oil blend was prepared by mixing coconut oil, safflower oil and soybean oil at a ratio of 2.5:1.1:0.8, to achieve a fatty acid profile comparable to that of HMF. Coconut, safflower and soybean oils were purchased from local retail outlets in Athens, Georgia. Coconut oil was melted to a liquid form at 40°C before mixing. The mixture was stirred vigorously to ensure uniform distribution then stored at 40±2°C to prevent re-crystallization of fat particles.

Interesterification of Triacylglycerol

Two similarly formulated lipid groups were prepared with each group containing two separate mixtures of tripalmitin and vegetable oil blends at different substrate molar ratios of 1:0.4 and 1:1, respectively. The mixtures were placed in screw-capped test tubes containing 3 ml n-hexane and Lipozyme RM IM enzyme (10 wt % of total reactants). The sample mixtures were incubated separately for 12 and 24 hr at 55 °C in an orbital shaking water bath at 200 rpm, giving rise to 4 (2×2) samples. All formulated lipids were
prepared in four replicates and chemically analyzed in duplicates. The enzyme and products were passed through a sodium sulfate column to stop the reaction and remove the enzyme from the reaction products, as described in Sahin et al. (2005a).

The reaction products were applied to TLC plates (20×20 cm) coated with silica gel G, and were developed in a TLC tank using petroleum ether/ethyl ether/acetic acid (80:20:0.5 v/v) as the development solvent as described in Sahin et al. (2005a). The bands were sprayed with 0.2 % 2,7-dichlorofluorescein in methanol and visualized under ultraviolet (UV) light. The TAG band produced was scrapped into a screw-cap test tube and extraction of the TAG from the band was carried out by twice vortexing the scrapped band with 3 ml hexane and centrifuging at 2000 rpm for 5 min. The solvent was evaporated in a 25 ml evaporator flask using a rotary evaporator at 60 °C. The four fat samples (from 2 molar ratios, 2 reaction times) produced from each replicate were then stored separately at −85 °C. This interesterification of TAGs was repeated four times.

Preparation of infant formula analog

Fresh caprine milk was pasteurized at 72 °C for 30 min and the fat was separated from the milk using a cream separator (Armfield FT 15, London, England). The resultant caprine skim milk was subdivided into four equal portions, in four replicates, and each portion was separately combined with one of the four prepared lipid group samples at a rate of 0.4 g fat per 10 g milk to produce four different infant formula analogs; as F1 (1:0.4 molar ratio of tripalmitin vs vegetable oil blend, esterified for 12 hr), F2 (1:1 molar ratio of tripalmitin vs vegetable oil blend, esterified for 12 hr), F3 (1:0.4 molar ratio of tripalmitin vs vegetable oil blend, esterified for 24 hr) and F4 (1:1 molar ratio of tripalmitin vs vegetable oil blend, esterified for 24 hr). These products were then freeze-
dried at a temperature of –40 °C and a pressure of 133×10^{-4} \text{kPa}, using a freeze-drier (Virtis Freeze Mobile 25, Gardiner, NY).

\textit{Fat extraction}

Lipids were extracted from the freeze-dried infant formula analogs using the procedure of Folch et al., (1957). One gram of the freeze-dried sample was homogenized with 12 ml of pre-chilled chloroform-methanol (2:1 \text{v/v}) containing 0.005 \% butylated hydroxy toluene. The sample tubes were centrifuged at 2000 rpm for 5 min to separate insoluble particles, and the solvent layer was passed through a sodium sulfate column. The solvent was evaporated in 50 ml evaporation flasks using a rotary evaporator at 60 °C, and the fat extracts were stored in a freezer (-85 °C).

\textit{Preparation of fatty acid methyl esters (FAME)}

The fat extracted was reconstituted with 2 ml chloroform-methanol and flushed with nitrogen using a nitrogen evaporator (N-EVAP Organomation, Model No. 111 Organomation, Berlin, MA). A 200 μl sub-sample was pipetted from each reconstituent and transferred to a screw-cap reaction tube. A 20 μl quantity of heptadecanoic acid was added to each tube to act as an internal standard. Excess solvent was evaporated from each tube after which 3 ml of 6 \% HCl in methanol was added, and the mixture was methylated by incubating at 75 °C for 2 hr in a pre-heated oven. Thereafter, the mixture was extracted twice with 2 ml hexane and 1 ml 0.1 M KCl, and centrifuged at 1000 rpm (400 x g) for 3 min, and the upper (hexane) layer was separated and combined for each sample, then passed through sodium sulfate column as described in Sahin et al. (2005a). The excess solvent was evaporated under nitrogen until about 1ml aliquot was obtained.
Quantification of fatty acids by GC

Fatty acid composition of the extracts was quantified by Agilent 6890N gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector (FID). Helium was the carrier gas and the gas flow rate was 1.7 ml/min. The oven temperature was initially held at 80°C for 3 min, and then programmed to 215°C for 10 min at a rate of 10°C/min and held isothermally for 20 min as described in Sahin et al. (2005a). The column used was a fused Silica Heliflex capillary column (Alltech-AT-225 30 mm × 0.25 mm × 0.25 μm film thickness; Deerfield, IL). The different amounts of FAME (mol %) were analyzed and integrated by an integrator (model G2070AA, Agilent Technologies, Palo Alto, CA) with reference to C17:0 as an internal standard.

Sn-2 position analysis of TAGs by pancreatic lipase

Fatty acids at the Sn-2 position were analyzed according to the method described in Sahin et al. (2005a). Quantities of 20 mg pancreatic lipase, 1 ml Tris buffer (pH 8.0), 0.25 ml bile salts (0.05 %) and 0.1 ml calcium chloride (2.2 %) were added to a test tube (25×200 mm) containing 0.1 g fat sample extracted from each of the infant formula analogs as described above. The sample reaction mixture was incubated at 40 °C in a water bath for 3 min. Then 1 ml of 6 M HCl and 1 ml diethyl ether were added, and the tube was centrifuged. The diethyl ether layer was evaporated under nitrogen stream (N-EVAP Organomation model No. 111) to the volume of about 200 μl.

A 200 μl aliquot was spotted unto a silica gel G TLC plate and developed in a TLC tank by using hexane/diethyl ether/acetic acid (50:50:1 v/v) as the developing solvent. The 2-monoacylglycerol (2-MAG) band produced was sprayed with 0.2 % 2,7-dichlorofluorescein in methanol and identified under UV light. The 2-monoolein standard (Sigma) was used to confirm the TLC separation of 2-MAG in the reaction products. The
2-MAG band was then scrapped-off into a screw-capped test tube, extracted twice with 1 ml of hexane, and fatty acid methyl esters were derivatized as aforementioned, and then analyzed by GC to evaluate the enzymatic interesterification of fatty acids at the sn-2 position of the TAG.

Statistical analysis

All data collected from the four experimental infant formula analogs in 4 replicates of enzymatic interesterification were analyzed by ANOVA for mean differences between treatments. The effects of substrate molar ratio, reaction times and their interactions were analyzed using the GLM procedure of SAS (SAS Institute, 1996).

RESULTS AND DISCUSSION

Fatty Acid Composition of Infant Formula Analog.

The fatty acid profiles and sn-2 fatty acid distribution of the three vegetable oils are shown in Table 4.1. Our data reveals that coconut oil was an important source of medium and long chain saturated fatty acids, while safflower and soybean oils are integral sources of polyunsaturated fatty acids, which are in agreement with Packard (1982). In light of sn-2 position fatty acids of the vegetable oils, coconut oil had 81 % as C12:0, safflower oil had 65 % as C18:1 and soybean oil had about 69 % as C18:2 fatty acid (Table 4.1).

The fatty acid profiles of the formulated goat milk analogs (F2, F3 and F4) were similar to that of human milk (α = 0.05) by consisting mainly of medium and long chain fatty acids, with unsaturated fatty acids ranging from oleic to trace amounts of arachidonic acid (Table 4.2). There were differences (P < 0.05 or 0.01) in fatty acid contents (mol %) in the goat milk infant formula analogs, with palmitic acid being
highest in F1 and F3 formulas, and oleic acid being the highest in F2 and F4. Oleic acid in human milk accounts for 38 % of total fatty acids, whereas other higher fatty acids include palmitic acid (23 %), linoleic acid (9 %) and myristic acid (8 %) (Table 4.2). These four major fatty acids in human milk account for about 78 % of total fatty acids, with oleic and palmitic acids contributing more than 75 % of the total. In the infant formula analogs, F2 had the closest similarity to HMF in this aspect followed by F4, F3 and F1 in that order. The oleic, palmitic, linoleic, and myristic fatty acids of these four formula analogs accounted for about 73.3 %, 75.8 %, 78.1 % and 86.6 % of their total fatty acids, respectively.

_Palmitic Acid Incorporation._

The fatty acids in F1 and F3 consisted predominantly of palmitic acid with F1 having the highest palmitic acid content (Table 4.2). The contents of palmitic acid in these samples, in comparison to the other two groups (F2 and F4), can be attributed to the high ratio of tripalmitin reacting with the vegetable oil blends, which subsequently resulted in a higher level of incorporation of the palmitic acid into the TAG of the samples. Although F2 and F4 had lower levels of palmitic acid incorporation, their mole percentage of palmitic acid (25.9 % and 28.2 %, respectively) were closer to that of human milk (23 %) in comparison with those of F1 (43.2 %) and F3 (32.7 %).

_Oleic and Stearic Acid Incorporation._

Oleic acid accounted for about 28.7 %, 31.7 %, 30.1 % and 31.8 % total fatty acids in F1, F2, F3 and F4, respectively, while stearic acid accounted for 6.4 %, 6.03 %, 6.4 % and 6.04 % of total fatty acids in the corresponding formula groups. These outcomes are similar to the oleic and stearic acid contents of human milk fat, which accounted for 38 % and 7 % of the total fatty acids, respectively. The higher oleic acid
incorporation of F2 and F4 can be explained by the higher content of vegetable oil in F2 and F4 in comparison with the other two analogs. Reaction time also influenced the oleic acid content of the formulated samples (Table 4.2). Increasing reaction time from 12 to 24 hrs also resulted in a subsequent increase in oleic acid contents of F3 and F4 compared to those of F1 and F2, which is in agreement with other previous reports (Fomuso and Akoh, 1997; Lee and Akoh, 1998; Sahin et al., 2005b).

Linoleic Acid Incorporation.

The respective linoleic acid contents (%) of the infant formula analogs F1, F2, F3 and F4 were 8.28, 9.72, 8.85 and 9.67 (Table 4.2). Linoleic acid is the main essential polyunsaturated fatty acid in human milk (Agostoni, 2003). The linoleic acid in human milk is around 9 % of total fatty acids, and it also makes up about 83 % of the polyunsaturated fatty acids in HMF (Figure 4.1). Although there was no significant difference (P > 0.05) in linoleic acid content between human milk and the formula analogs, F2 had the highest mean linoleic acid, while F1 had the lowest (Figure 4.1 and Table 4.2). On the other hand, linoleic acid contributed about 85 % of the polyunsaturated fatty acids in these infant formula analogs. In addition, there were increases in linoleic acid levels of the formula Analogs as substrate molar ratio and reaction times increased (Figure 4.1). The F2 had the greater linoleic acid incorporation after the esterification reaction (Table 5.2), which is due to its high substrate molar ratio as well as to its higher reaction time in comparison with F4 and the other two formula groups.

Polyunsaturated vs Saturated Acid Ratio

The polyunsaturated to saturated fatty acid ratios (P/S) of the formulated analogs are comparable to that of human milk (Figure 4.2). The saturated fatty acid levels of the
formulae is of considerable importance due to certain health concerns from the influence of saturated fatty acids on low density lipoproteins in human metabolism (Shepherd et al., 1980; Spady and Dietschy, 1985). Koletzko and Bremer (1989) indicated that most manufacturers of infant formulae aim at a P/S ratio close to 0.2-0.5. The P/S ratio in HMF is about 0.23 (Jensen, 1989), which is not significantly (P > 0.05) different from those of the infant formulae in this study.

Monounsaturated Fatty Acid (MUFA) vs Polyunsaturated Fatty Acid (PUFA) Content

The MUFA and PUFA contents, and their ratios in the infant formula analogs and human milk are shown in Figure 4.3. HMF contains about 39% MUFA and 10.8% PUFA, where both account for approximately 50% of total milk lipids. These fat moieties in F2 analog was 43.3% of total lipids, which appeared to have better resemblance to those of human milk in comparison with other formulated fats. The F1, F3 and F4 have slightly lower values of total MUFA and PUFA contents with F1 having the lowest value. These values may have resulted from the high rate of tripalmitin inclusion in the F1 Analog sample, in comparison to other formulated Analog samples.

Oleic to Palmitic Acid Ratio

The oleic to palmitic acid ratio (OPR) of the formulated infant formulae relative to that of human milk is depicted in Figure 4.4. In human milk, oleic acid has the highest content followed by palmitic acid, resulting in a 1.65 ratio of these two fatty acids as. The OPR of F2 and F4 infant formulae were more similar to that of human milk, whereas OPR values of F1 and F3 groups were relatively low. The reason for a lower OPR in F1 and F3 were be actually attributable to their levels of palmitic acid incorporation at the substrate molar ratio at which they were reacted. According to Xu (2000), modified lipids with OPR values of approximately 1.2 are desirable for infant food formulations.
Sn-2 Positional Analysis with Pancreatic Lipase

The sn-2 positional fatty acid profiles of the infant formula analogs are shown in Table 4.3. The four formulated analogs contained sn-2 positional fatty acids profile in descending order of palmitic acid, oleic acid, linoleic acid, lauric acid, myristic acid and stearic acid. These fatty acid profiles of the formulated milks were closely similar to human milk. These formula analogs revealed a high percentage of palmitic acid at the sn-2 position with F4 containing the highest (66.4 %) and F2 the lowest (63.7 %). The high incorporation of palmitic acid at the sn-2 position of F3 and F4 may have been due to their longer reaction time in comparison to F1 and F2 (Table 4.3). The higher palmitic acid content of F1 compared to F2 would be also attributable to the high substrate molar ratio of F1, which enabled better retention of palmitic acid on the sn-2 position of the TAG backbone. However, there was no significant difference in palmitic acid content among the four formula groups.

The oleic acid distribution at the sn-2 position of F2 and F4 samples (Table 4.3) appears to be better simulated to that of human milk in comparison to those of F3 and F1. The higher substrate molar ratio of F2 and F4 samples might have resulted in subsequent increases in oleic acid incorporation. These results are in agreement with that of Sahin et al. (2005b), indicating that substrate molar ratio, temperature and time have positive effects on oleic acid incorporation with lypozyme RM IM. The incorporation level of linoleic acid at the sn-2 position in the infant formula analogs ranged from 8.37 % to 8.93 %. These values are higher than the sn-2 linoleic acid content of human milk (7.3 %).
CONCLUSIONS

Lipozyme RM IM enzyme was used in the interesterification because of its sn-1,3-specificity, that would result in incorporation of unsaturated fatty acids like oleic and linoleic acids at these specific sn-1,3 positions of the TAG backbone. Previous literature (Quinlan et al., 1995) indicates that unsaturated fatty acids at these positions would provide maximum fat and calcium absorption in infants.

The formulated caprine milk infant formula analogs produced in this study had similarities to human milk in terms of their fatty acid content and acyl distribution on the glycerol backbone. The F2 infant formula, produced with fat interesterified at 55 °C for 12 hr with a 1:10 molar ratio of tripalmitin vs vegetable oil blend (2.5 coconut, 1.1 safflower and 0.8 soybean oil), had better resemblance to human milk than did the other formulas under the conditions used for formulations of caprine milk in this study.

ACKNOWLEDGEMENT

This research was supported by the grants of SARE (Southern Region Research and Education)/USDA No. RD309-032/1575557, and USDA 1890 Capacity Building grant No. 2001-38814-11388. The authors appreciate Schauston Miller for the collection of goat milk at the Georgia Small Ruminant Research and Extension Center, Fort Valley, GA.

LITERATURE CITED


SAS 1996. The SAS program for windows. SAS Institute, Cary, NC.


Table 4.1. Fatty acid and sn-2 positional fatty acid profiles of vegetable oils (mol % /100 g)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Coconut oil</th>
<th>Safflower oil</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA Profile</td>
<td>Sn-2 Profile</td>
<td>FA Profile</td>
</tr>
<tr>
<td>C8:0</td>
<td>15.62±0.09</td>
<td>0.64±0.05</td>
<td>-</td>
</tr>
<tr>
<td>C10:0</td>
<td>10.27±0.03</td>
<td>2.73±0.11</td>
<td>-</td>
</tr>
<tr>
<td>C12:0</td>
<td>50.57±0.12</td>
<td>81.01±0.47</td>
<td>-</td>
</tr>
<tr>
<td>C14:0</td>
<td>12.92±0.08</td>
<td>9.78±0.25</td>
<td>-</td>
</tr>
<tr>
<td>C16:0</td>
<td>3.27±0.04</td>
<td>0.87±0.08</td>
<td>9.22±0.15</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.44±0.01</td>
<td>0.13±0.03</td>
<td>1.96±0.05</td>
</tr>
<tr>
<td>C18:1</td>
<td>1.29±0.08</td>
<td>3.47±0.06</td>
<td>74.01±0.20</td>
</tr>
<tr>
<td>C18:2</td>
<td>0.34±0.01</td>
<td>1.40±0.02</td>
<td>14.82±0.11</td>
</tr>
<tr>
<td>C18:3</td>
<td>-</td>
<td>-</td>
<td>Tr</td>
</tr>
<tr>
<td>C20:1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Tr: Trace (< 0.01)
Table 4.2. Fatty acid profiles (mol%) of Infant formula analogs and human milk.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>GM</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>HM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>2.24±0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C6:0</td>
<td>2.43±0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C8:0</td>
<td>2.71±0.06a</td>
<td>1.11±0.81a</td>
<td>2.49±0.41b</td>
<td>1.54±1.46a</td>
<td>1.75±0.67a</td>
<td>-</td>
</tr>
<tr>
<td>C10:0</td>
<td>9.89±0.20b</td>
<td>2.14±0.58b</td>
<td>3.65±0.13a</td>
<td>3.26±1.23a</td>
<td>3.34±0.26a</td>
<td>2a</td>
</tr>
<tr>
<td>C12:0</td>
<td>4.89±0.11a</td>
<td>3.8±2.72a</td>
<td>7.1±0.53b</td>
<td>8.9±2.34b</td>
<td>10.9±0.99b</td>
<td>7b</td>
</tr>
<tr>
<td>C14:0</td>
<td>9.69±0.02a</td>
<td>5.13±0.61a</td>
<td>7.04±0.15a</td>
<td>6.89±3.22a</td>
<td>6.8±0.10a</td>
<td>8a</td>
</tr>
<tr>
<td>C16:0</td>
<td>30.1±0.19a</td>
<td>43.2±6.31b</td>
<td>25.9±1.02a</td>
<td>32.7±6.36a</td>
<td>28.2±1.65ab</td>
<td>23a</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.59±0.05a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3a</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.00±0.51b</td>
<td>6.4±0.15a</td>
<td>6.03±0.05a</td>
<td>6.4±0.07a</td>
<td>6.04±0.10a</td>
<td>7ab</td>
</tr>
<tr>
<td>C18:1</td>
<td>18.2±0.42a</td>
<td>28.69±1.57ab</td>
<td>31.69±0.27b</td>
<td>30.14±1.66b</td>
<td>31.84±0.42b</td>
<td>38ab</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.33±0.09a</td>
<td>8.28±0.73b</td>
<td>9.72±0.20b</td>
<td>8.84±0.94b</td>
<td>9.67±0.19b</td>
<td>9b</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.37±0.04a</td>
<td>1.32±0.08b</td>
<td>1.44±0.02b</td>
<td>1.36±0.13b</td>
<td>1.57±0.04b</td>
<td>1b</td>
</tr>
<tr>
<td>C20:1</td>
<td>-</td>
<td>Tr</td>
<td>Tr</td>
<td>0.15±0.03a</td>
<td>Tr</td>
<td>1b</td>
</tr>
<tr>
<td>C20:4</td>
<td>-</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>0.8</td>
</tr>
</tbody>
</table>


F1: Infant formula Analog produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio) and esterified for 12 hr.

F2: Infant formula Analog produced with tripalmitin and vegetable oil blend (1:1 molar ratio) and esterified for 12 hr.

F3: Infant formula Analog produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio) and esterified for 24 hr.

F4: Infant formula Analog produced with tripalmitin and vegetable oil blend (1:1 molar ratio) and esterified for 24 hr.

Tr: Trace (< 0.01)

a,b,c Means with different superscript in a same row are different (P<0.05 or P<0.01).
Table 4.3. Sn-2 positional distribution of fatty acids (mol %) in goat milk, infant formula analogs and human milk.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>GM</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>HM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>-</td>
<td>0.55±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67±0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>C10:0</td>
<td>11.18±2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.63±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C12:0</td>
<td>7.54±0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.53±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.58±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.66±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.47±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C14:0</td>
<td>14.8±4.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.06±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.51±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.25±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.28±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:0</td>
<td>40.3±5.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.6±1.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>63.7±2.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>66.0±0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>66.4±0.36&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>58.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C18:0</td>
<td>26.14±3.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.55±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.70±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1</td>
<td>-</td>
<td>12.19±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.42±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.28±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.87±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2</td>
<td>-</td>
<td>8.66±0.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.37±0.79&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.45±0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.93±0.43&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:3</td>
<td>-</td>
<td>0.33±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.51±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:1</td>
<td>-</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>0.7</td>
</tr>
<tr>
<td>C20:4</td>
<td>-</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*HM: Human milk; Source: Jensen (1989); GM: Goat Milk

F1: Infant formula Analog produced with tripalmitin and vegetable oil blend
(1:0.4 molar ratio, 12hrs).

F2: Infant formula Analog produced with tripalmitin and vegetable oil blend
(1:1 molar ratio, 12hrs).

F3: Infant formula Analog produced with tripalmitin and vegetable oil blend
(1:0.4 molar ratio, 24hrs).

F4: Infant formula Analog produced with tripalmitin and vegetable oil blend
(1:1 molar ratio, 24hrs).

Tr: Trace (< 0.01)

<sup>a,b,c</sup>Means with different superscript in a same row are different (P<0.05 or P<0.01).
Figure 4.1. Percent linoleic acid content in PUFA and total fat of human milk and infant formulae. GM: Goat milk. F1: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12hrs). F2: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12hrs). F3: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24hrs). F4: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24hrs). HM: Human milk. Formulae were interesterified at 55°C, enzyme amount was 10 wt % of total substrates. Incubation was at 200 rpm in n-hexane. F1, F2, F3, and F4 are not significantly different from HM, while GM is significantly different from HM (P< 0.05). Sample number (n) = 8.
% C18:2 in total fat
% C18:2 in PUFA
Figure 4.2. Polyunsaturated to saturated fatty acid ratio of human milk and infant formulae. GM: Goat milk. F1: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12hrs). F2: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12hrs). F3: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24hrs). F4: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24hrs). HM: Human milk. Formulae were interesterified at 55°C, enzyme amount was 10 wt % of total substrates. Incubation was at 200 rpm in n-hexane. F1, F2, F3, and F4 are not significantly different from HM, while GM is significantly different from HM (P< 0.05). Sample number (n) = 8.
Figure 4.3. Percentage totals and ratios of monounsaturated and polyunsaturated fatty acid content of human milk and infant formulae. GM: Goat milk. F1: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12hrs). F2: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12hrs). F3: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24hrs). F4: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24hrs). HM: Human milk. Formulae were interesterified at 55°C, enzyme amount was 10 wt % of total substrates. Incubation was at 200 rpm in n-hexane. F1, F2, F3, and F4 are not significantly different from HM, while GM is significantly different from HM (P< 0.05). Sample number (n) = 8.
Figure 4.4: Oleic acid to palmitic acid ratio (OPR) of human milk and infant formulae.

GM: Goat milk. F1: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12hrs). F2: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12hrs). F3: Infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24hrs). F4: Infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24hrs). HM: Human milk. Formulae were interesterified at 55°C, enzyme amount was 10 wt % of total substrates. Incubation was at 200 rpm in n-hexane. F2 and F4 are not significantly different from HM, while GM, F1, and F3 are significantly different from HM (P< 0.05). Sample number (n) = 8.
CHAPTER 5

ENZYMATIC PRODUCTION OF INFANT MILK FAT ANALOGS CONTAINING PALMITIC ACID: OPTIMIZATION OF REACTIONS BY RESPONSE SURFACE METHODOLOGY


Reprinted here with permission of the publisher.
ABSTRACT

Infant milk fat analogues resembling human milk fat were synthesized by an enzymatic interesterification between tripalmitin, coconut oil, safflower oil and soybean oil in hexane. Commercially immobilized 1,3-specific lipase, Lipozyme RM IM, obtained from Rhizomucor miehei was used as a biocatalyst. The effects of substrate molar ratio, reaction time, and incubation temperature on the incorporation of palmitic acid at the sn-2 position of the triacylglycerols were investigated. A central composite design with five levels and three factors consisting of substrate ratio, reaction temperature, and incubation time was employed to model and optimize the reaction conditions using response surface methodology. A quadratic model using multiple regressions was then obtained for the incorporation of palmitic acid at the sn-2 positions of glycerols as the response. The coefficient of determination ($R^2$) value for the model was 0.845. The incorporation of palmitic acid appeared to increase with decrease in substrate molar ratio and increase in reaction temperature, and optimum incubation time occurred at 18 h. The optimal conditions generated from the model for the targeted 40% palmitic acid incorporation at the sn-2 position were 3 mol/mol, 14.4 h and 55°C; and 2.8 mol/mol, 19.6 h and 55°C for substrate ratio (moles total fatty acid/mol tripalmitin), time, and temperature, respectively. Infant milk fat containing fatty acid composition and sn-2 fatty acid profile similar to human milk fat was successfully produced. The fat analogs produced under optimal conditions had total and sn-2 positional palmitic acid levels comparable to that of human milk fat.

KEY WORDS: Enzymatic interesterification, infant milk fat, response surface methodology, sn-1,3-specific lipase.
INTRODUCTION

Human milk is the best source of nutrients for infants (Mehraud et al., 1990) and contains about 4-5% fat, which supplies the highest fraction of the infants’ required dietary energy (Yang et al., 2003). The triacylglycerols (TAGs) in human milk fat (HMF) constitutes about 98% of total fat and are dominated by palmitic acid (23%), which also dominates the fatty acids at the sn-2 position (40-58%) of the glycerol backbone (USDA, 1976; Jensen, 1989; Xu, 2000).

In HMF (unlike vegetable oils, ruminant milks and infant formulas), the sn-1 and sn-3 positions are occupied by unsaturated fatty acids (Innis et al., 1995; Xu, 2000), where the location of palmitic acid at the sn-2 position of TAGs in HMF increases the absorption of fatty acids in the lumen of infants and decreases the loss of calcium in their feaces (Quinlan et al., 1995; Kennedy et al., 1999). This is due to the preservation of the sn-2 positional fatty acid during digestion, absorption, and biosynthesis of TAGs in the intestinal wall.

Milk can be modified for infant feeding by redesigning its physical, chemical, and nutritional properties. Modified lipids resembling the TAGs of human milk fat can be produced by interesterification reactions using sn-1,3-specific lipase as the biocatalyst (Sahin et al., 2005b). Use of this lipase gives high selectivity and mimics the natural pathways of metabolic processes (Akoh et al., 2002).

Preliminary studies in our laboratory have revealed that combinations of coconut, safflower and soybean oils in a 2.5:1.1:0.8 ratio, have a fatty acid profile similar to that of HMF (Maduko et al., 2005, 2007). Coconut oil is a good source of medium and long chain saturated fatty acids, whereas safflower and soybean oils are two sources of polyunsaturated fatty acids that are suitable for infant milk formulation (Packard, 1982).
However, the positions of fatty acids in TAGs of vegetable oils are different from those of HMF. It is beneficial that the TAGs of infant formulas produced with vegetable oils be modified to simulate that of HMF for better absorption. Previous studies by Maduko et al. (2007) have revealed that enzymatic interesterification of tripalmitin with vegetable oil blend containing coconut, safflower and soybean oils with Lipozyme RM IM can be successful in the simulation of infant milk fat to HMF. Lipozyme RM IM is used in interesterification reactions because of its sn-1,3-specificity, which results in the incorporation of fatty acids at the sn-1,3 positions of the TAG backbone.

The objective of this study therefore is to model and optimize the incorporation of palmitic acid into a blend of coconut, safflower and soybean oils (2.5:1.1:0.8 v/v/v) using response surface methodology (RSM) to form infant milk fat analogue. RSM consists of a set of mathematical and statistical methods developed for modeling phenomena and finding combinations of a set of a number of experimental factors that will lead to optimal response (Lumor and Akoh, 2005). The model developed can then be employed in further studies for up-scaling and physical characterization of the infant formula fat. Parameters studied were substrate molar ratio (moles total fatty acid/mol tripalmitin), temperature and time of reaction. The reactions were optimized with respect to palmitic acid incorporation at the sn-2 position of the glycerols.

MATERIALS AND METHODS

Chemicals

Tripalmitin (glycerol tripalmitate, minimum purity of 85%) and porcine pancreatic lipase (type II, crude) were purchased from Sigma Chemical Co. (St. Louis, MO). Immobilized 1,3-specific lipase, Lipozyme RM IM, was purchased from Novo Nordisk A/S
Bagsvaerd, Denmark). Organic solvents and thin-layer chromatography (TLC) plates were purchased from J.T. Baker Chemical Co. (Phillisburg, NJ) and Fisher Scientific (Fair Lawn, NJ), respectively. All solvents and reagents used for sample analysis were chromatographic or analytical grade.

**Preparation of Vegetable Oil Blend.**

Vegetable oil blend was prepared by mixing coconut oil, safflower oil and soybean oil at a ratio of 2.5:1.1:0.8, to achieve a fatty acid profile comparable to HMF as described by Maduko et al. (2005, 2006b). Coconut oil was melted to a liquid form at 40°C before mixing. The mixture was stirred vigorously to ensure uniform distribution then stored at –85°C until needed for further analysis.

**Experimental Design for RSM Study**

The optimal conditions for palmitic acid incorporation at the sn-2 position with respect to substrate molar ratio, incubation temperature and reaction time were determined using RSM as described by Lumor and Akoh (2005), Sahin et al. (2005a, 2006). A three-variable five-level central composite design (CCD) was used to generate factor combinations (Montgomery, 1997). The variables chosen were substrate molar ratio (\(\text{Mr}: \text{moles total fatty acid/mol tripalmitin}\)), incubation time (\(\text{t}: \text{in hours}\)) and reaction temperature (\(\text{T}: \text{in } ^\circ\text{C}\)), and the response sought was mol% incorporation of palmitic acid at the sn-2 position of TAG. The experimental design containing the independent variables is presented in Table 1. A total of 34 runs were generated and experiments at each design point were carried out in duplicate. The design consisted of 6 factorial points, 12 axial points (2 axial points on the axis of each design variable), and 16 center points. The data developed from the design in Table 5.1 were used to fit a second order polynomial function as follows:
\[ Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon_{ij} \]

Where:

Y = The response (% incorporation of palmitic acid at the sn-2 position of the TAG)

\( \beta_o \) = Constant

\( \beta_i \) = Linear (first order model)

\( \beta_{ii} \) = Quadratic (second order model)

\( \beta_{ij} \) = Interaction term coefficients

\( X_i \) and \( X_j \) = Independent variables (enzyme, vegetable oil blend)

\( \varepsilon_{ij} \) = Error term

**Interesterification Reaction**

The interesterification mixture, containing 3 mL hexane, and a mixture of tripalmitin and vegetable oils at different substrate Mr ranging from 1 to 12 determined by the RSM design (Table 1) were placed in screw-capped test tubes containing immobilized lipase, Lipozyme RM IM (10 wt% of total reactants). The mixtures were incubated in an orbital shaking water bath at 200 rpm. All reactions were performed and analyzed in duplicate. The enzyme and products were passed through a sodium sulfate column to stop the reaction and remove the enzyme from the reaction products, as described by Sahin et al. (2005b).

**Analysis of Products***
The reaction products were applied to thin layer chromatography (TLC) plates (20 x 20 cm) coated with silica gel G, and were developed in a TLC tank using petroleum ether: ethyl ether: acetic acid (80:20:0.5 v/v/v) as the development solvent as described by Sahin et al. (2005b). The separated bands were sprayed with 0.2% 2,7-dichlorofluorescein in methanol and visualized under ultraviolet (UV) light. The TAG band containing the new TAG product and un-reacted TAG, was scrapped into a screw-capped test tube, and methylated with 3 ml of 6% HCl in methanol at 75°C for 2 h. The fatty acid methyl esters (FAME) produced were extracted twice with 2 ml of hexane and dried over an anhydrous sodium sulfate column according to the method described by Jennings and Akoh (1999).

**Fatty Acid Compositional Analysis**

Fatty acid composition of the scrapped TAG bands was quantified by gas chromatography (Model 6890N GC, Agilent, Palo Alto, CA) equipped with a flame ionization detector. Helium was the carrier gas and the gas flow rate was 1.7 mL/min. The oven temperature was initially held at 80°C for 3 min, and then programmed to 215°C for 10 min at the rate of 10°C/min, and held isothermally for 20 min as described by Sahin et al. (2005a). The column used was a fused silica Heliflex capillary column (Alltech-AT-225 30 mm × 0.25 mm × 0.25 μm film thickness; Deerfield, IL). The different amounts of FAME (mol%) were analyzed and integrated by an integrator (model G2070AA, Agilent) with reference to C17:0 as an internal standard.

**Sn-2 Fatty Acid Position Analysis of TAGs by Pancreatic Lipase.**

Fatty acids at the sn-2 position were analyzed according to the method described by Sahin et al. (2005a). Twenty milligrams of pancreatic lipase, 1 mL Tris buffer (pH 8.0), 0.25 mL bile salts (0.05%) and 0.1 mL calcium chloride (2.2%) were added to a test
tube (25×200 mm) containing 0.1 g fat sample extracted from each of the infant formula analogues as described above. The sample reaction mixture was incubated at 40°C in a water bath for 3 min. Then 1 mL of 6 M HCl and 1 mL diethyl ether were added, and the tube was centrifuged. Diethyl ether layer was evaporated under nitrogen stream (N-EVAP Organomation model No. 111) to a final volume of about 200 μL.

A 200 μL aliquot was spotted unto a silica gel G TLC plate and developed in a TLC tank by using hexane: diethyl ether: acetic acid (50:50:1 v/v/v) as the developing solvent. The plate was sprayed with 0.2% 2,7-dichlorofluorescein in methanol and bands visualized under UV light. The 2-monoolein standard (Sigma) was used to confirm the TLC separation of 2-monoacylglycerol (2-MAG)s in the reaction products. The 2-MAG band was then scrapped-off into a screw-capped test tube, extracted twice with 1 mL of hexane, and fatty acid methyl esters were prepared as aforementioned, and then analyzed by GC to evaluate the enzymatic incorporation of fatty acids at the sn-2 position of the TAG.

Statistical Analysis.

Regression analysis, response surfaces, and statistical significance were done using MODDE 5.0 Software (Umetrics, Umea, Sweden).

RESULTS AND DISCUSSION

Model Fitting

Modified lipids enriched with palmitic acid were produced by enzymatic interesterification of tripalmitin with vegetable oil blend. The incorporation level of palmitic acid at the sn-2 position of the TAG in the infant formula fat analogues ranged
from 7.9 to 55.3%, and the targeted incorporation level was 40-55%, which is the range found in HMF (Jensen, 1989).

A three-factor, five-level CCD was used for the reactions and the design points and responses are given in Table 5.1. Quadratic models were obtained for the incorporation of palmitic acid at the sn-2 position (response) by multiple linear regression. The regression coefficients ($\beta$) and significance (P-) values were calculated based on the results in Table 5.1 and are given in Table 5.2. Time of reaction appeared to have a negative impact on palmitic acid incorporation (Table 5.2), whereas substrate molar ratio appeared to have the most significant effect on palmitic acid incorporation followed by the temperature of reaction.

The second order parameters of incubation time ($t \times t$), substrate molar ratio ($Mr \times Mr$), and reaction temperature ($T \times T$) all had significant positive effects, while the interaction terms of reaction temperature and incubation time ($T \times t$); reaction temperature and substrate molar ratio ($T \times Mr$); and incubation time and substrate molar ratio ($t \times Mr$), had no significant effects on the response and were therefore excluded from the model. $R^2$, the fraction of the variation of the response explained by the model, was 0.845 and $Q^2$, the fraction of the variation of the response that can be predicted by the model, was 0.673, while the value of $R^2$ adjacent was 0.787.

The normal prediction plot showed a linear distribution (Figure 5.1) and the observed vs. prediction plot (Figure 5.2) appeared to have a linear distribution as well. The model had a strong reproducibility (0.948) and the P-value of the multiple regression was less than 0.001. The model equation can therefore be written as:

$$\text{Incorporation} = 22.5796 - 3.58481 T - 7.3129 Mr - 3.90844T^2 - 3.39591 t^2 + 3.49712 Mr^2.$$
All coefficients were highly significant \( (P < 0.05) \) except for the first order parameter time, and the interaction terms (Table 5.3), which appeared to be significant only at \( P \)-value 0.5 or higher. However, the second order parameter time was highly significant \( (P < 0.005) \) and was therefore included in the model.

**Optimization of the Reaction**

From the model equation, it appears that the incorporation of palmitic acid at the sn-2 position was affected to a large extent by the first- and second-order variables (Table 5.3). Palmitic acid incorporation would therefore be related to the parameters that include the first and second-order polynomials, which may lead to more than one solution (Xu et al., 2000; Lumor and Akoh, 2005). A good way to analyze the relationships between the responses, parameters, and any interactions that may exist within is to analyze the contour plots (Lumor and Akoh, 2005) for palmitic acid incorporation. For the contour plots construction, the variable with the greatest effect on the response was placed on the y-axis, the second on the x-axis, whereas the variable with the least effect was held constant as described by Lumor and Akoh (2005). The response plots obtained by the interaction of the three parameters on the incorporation of palmitic acid at the sn-2 position of the TAG are given in Figure 5.3. The third variable \( (Mr; \text{ levels 1 to 3}) \) was used as intermediate level for drawing the contour plots. There appeared to be a general increase in incorporation level as the three variables were increased. Depending on the level of substrate molar ratio, incorporation level increased or decreased with increasing temperature at constant time. These results are similar to those reported by Lumor and Akoh (2005), and Sahin et al. (2005), where higher reaction temperatures increased the rate of productive collisions between reactants and enzyme, thereby giving rise to
increased reaction rate (Paivi et al., 2000). On the other hand, higher temperatures also accelerate the rate of enzyme inactivation, and thereby lead to decrease in incorporation levels (Dordick, 1989). This is attributable to the increased rate of enzyme denaturation surpassing the rate of productive collisions between enzyme and reactants, therefore leading to an overall decrease in reaction rate (Paivi et al., 2000; Lumor and Akoh, 2005).

The optimum incubation time remained constant (18 h) with increase in Mr, whereas palmitic acid incorporation increased with decreases in Mr and T. The targeted 40-55% incorporation of palmitic acid was observed at reaction temperatures of 55-61°C (Figure 5.3a), 55-61°C (Figure 5.3b) and 55-58°C (Figure 5.3c), thus varying with the substrate molar ratio. The targeted range for palmitic acid incorporation was obtained at all incubation time levels in Figures 5.3a and 5.3b, while this target range was observed between 16.5 and 21.5 h in Figure 5.3c. Optimal palmitic acid incorporation (54.3%) occurred at Mr 1, 16-21 h, and 55-57°C (Figure 5.3a).

The conditions for the targeted palmitic acid incorporation (40-55%) at the sn-2 position of TAG were generated by the optimizer function of the MODDE 5.0 software. The optimal conditions were calculated using the generated model equation and the targeted palmitic acid incorporation (40-55%) at the sn-2 position as the criteria, and are given in Table 5.4. The targeted range of palmitic acid incorporation can be obtained with Mr of 1 to 3, with maximum incorporation obtained at Mr 1 (Table 5.4). The lower range for the target palmitic acid incorporation (40%) can be obtained with Mr 3 at 55°C and 14.4 h, which happens to be the optimal incorporation conditions at this substrate molar ratio. These conditions were chosen as optimum because they meet the target level of 40 to 55% incorporation and also appear to be feasible for large-scale production of the analog due to lower amounts of tripalmitin required to achieve up to 40% incorporation.
These conditions would therefore minimize the cost of reaction materials and economize energy requirements during production, unlike that for Mr 1 and 2.

Verification of the Models

To verify the model, experiments were performed using the conditions specified for five chosen regions from the contour plot as described by Lumor and Akoh (2005). A chi-square test (Table 5.5) indicated no significant difference between the observed and predicted values. The chi-square value (2.6237) was smaller than the cutoff point (9.488) at $\alpha_{0.05}$ and 4df.

Enzymatic interesterification reactions were performed in test tubes using the chosen optimal conditions obtained with RSM to further verify the model as described by Sahin et al. (2005b). Table 5.6 shows the results of the determination of total fatty acid composition and of the fatty acids at the sn-2 position of the resulting infant formula fat analogs. The fatty acid profile of the infant formula fat analog appeared to have a resemblance to the saturated fatty acids of HMF, which contains 23% palmitic, 2% capric, 8% lauric, and 7% stearic acid. The experimental values for palmitic acid incorporation at the sn-2 position appeared similar to the values predicted from the model. These values resemble the sn-2 fatty acid profile of HMF in which most of the saturated fatty acids, especially palmitic acid (40-60%) are at the sn-2 position and unsaturated fatty acids mainly at the sn-1 and sn-3 positions (Innis et al., 1995; Xu, 2000).

CONCLUSION

The position of palmitic acid in the glycerol backbone of HMF affects fatty acid absorption, calcium absorption and use of dietary energy (Sahin et al., 2005). In the
production of infant formula fat analogue, it is therefore necessary to simulate the fatty acid structure of HMF.

The determined model successfully optimized the enzymatic reaction conditions and predicted the incorporation level of palmitic acid in the infant milk fat analog. The optimum conditions generated for the target 40-55 % palmitic acid incorporation were at a molar ratio of 3, reaction temperature of 55°C, and incubation time of 14.4 h. These conditions appear to be both feasible and economical for large-scale production. The fatty acid profile of the infant formula fat analogue produced using these optimum conditions, showed a palmitic acid content of 24.6%, which is comparable to that of HMF (23%). Furthermore, the sn-2 fatty acid profile of the infant formula fat analog consists of 40.8% palmitic acid, which is within the targeted range of 40-60% palmitic acid incorporation found in HMF.

The second-order polynomial model, satisfactorily expressed the relationship between temperature of reaction, time of incubation, substrate molar ratio, and incorporation of palmitic acid at the sn-2 position of the vegetable oil blend used in this study. This model has a high reproducibility and can be used to design large-scale incorporation of palmitic acid into the vegetable oil blend for infant milk fat analogue production, whereby manufacturers can determine the level of incorporation desired, by increasing or decreasing the molar ratio of the substrates, while maintaining the reaction temperature and incubation time at the optimum levels.

LITERATURE CITED


Maduko, C.O., C.C. Akoh, and Y.W. Park. 20067. Enzymatic interesterification of
tripalmitin with vegetable oil blends to produce caprine milk infant formula

Maduko, C. O., C. C. Akoh, and Y. W. Park. 2006b. Comparison of the fatty acid
distributions among different vegetable oil blends toward infant milk formulation.
Savoy, IL.

milk fat to simulate fatty acid profile of human milk. 2005 IFT Annual Mtg.,
Abstr. 31-9. Institute of Food Technologists, Chicago, IL.

Megraud, F., G. Boudara, K. Bessand, S. Bensid, F. Dabis, R. Soltana, and M.
Touhahmi. 1990. Incidence of bacterial infection in infants in Western Algeria

John Wiley and Sons, New York.

Packard, V. S. 1982. Human milk and infant formula. Pages 140-175 in Infant formula
composition, formulation and processing. Food science and technology, a series
Press, New York.


Table 5.1: Experimental settings of the factors and the responses used for the optimization of the reaction by central composite design (CCD) experiments\(^a\)

<table>
<thead>
<tr>
<th>Exp No</th>
<th>T</th>
<th>t</th>
<th>Mr</th>
<th>Mol% C16:0 at sn-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>12</td>
<td>4</td>
<td>26.265</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>12</td>
<td>4</td>
<td>16.925</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>24</td>
<td>4</td>
<td>27.95</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>24</td>
<td>4</td>
<td>13.35</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>12</td>
<td>10</td>
<td>13.32</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>12</td>
<td>10</td>
<td>9.365</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>24</td>
<td>10</td>
<td>13.22</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>24</td>
<td>10</td>
<td>7.945</td>
</tr>
<tr>
<td>9</td>
<td>51.59</td>
<td>18</td>
<td>7</td>
<td>13.72</td>
</tr>
<tr>
<td>10</td>
<td>68.41</td>
<td>18</td>
<td>7</td>
<td>10.085</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>7.908</td>
<td>7</td>
<td>16.735</td>
</tr>
<tr>
<td>12</td>
<td>60</td>
<td>28.092</td>
<td>7</td>
<td>16.94</td>
</tr>
<tr>
<td>13</td>
<td>60</td>
<td>18</td>
<td>1.954</td>
<td>55.26</td>
</tr>
<tr>
<td>14</td>
<td>60</td>
<td>18</td>
<td>12.046</td>
<td>18.465</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>18</td>
<td>7</td>
<td>19.63</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>18</td>
<td>7</td>
<td>23.295</td>
</tr>
<tr>
<td>17</td>
<td>60</td>
<td>18</td>
<td>7</td>
<td>20.855</td>
</tr>
<tr>
<td>18</td>
<td>55</td>
<td>12</td>
<td>4</td>
<td>26.755</td>
</tr>
<tr>
<td>19</td>
<td>65</td>
<td>12</td>
<td>4</td>
<td>19.22</td>
</tr>
<tr>
<td>20</td>
<td>55</td>
<td>24</td>
<td>4</td>
<td>29.44</td>
</tr>
<tr>
<td>21</td>
<td>65</td>
<td>24</td>
<td>4</td>
<td>11.78</td>
</tr>
<tr>
<td>22</td>
<td>55</td>
<td>12</td>
<td>10</td>
<td>11.8</td>
</tr>
<tr>
<td>23</td>
<td>65</td>
<td>12</td>
<td>10</td>
<td>9.775</td>
</tr>
<tr>
<td>24</td>
<td>55</td>
<td>24</td>
<td>10</td>
<td>13.55</td>
</tr>
<tr>
<td>25</td>
<td>65</td>
<td>24</td>
<td>10</td>
<td>7.935</td>
</tr>
<tr>
<td>26</td>
<td>51.59</td>
<td>18</td>
<td>7</td>
<td>25.95</td>
</tr>
<tr>
<td>27</td>
<td>68.41</td>
<td>18</td>
<td>7</td>
<td>11.095</td>
</tr>
<tr>
<td>28</td>
<td>60</td>
<td>7.908</td>
<td>7</td>
<td>15.91</td>
</tr>
<tr>
<td>29</td>
<td>60</td>
<td>28.092</td>
<td>7</td>
<td>17.065</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>18</td>
<td>1.954</td>
<td>51.25</td>
</tr>
<tr>
<td>31</td>
<td>60</td>
<td>18</td>
<td>12.046</td>
<td>19.68</td>
</tr>
<tr>
<td>32</td>
<td>60</td>
<td>18</td>
<td>7</td>
<td>24.615</td>
</tr>
<tr>
<td>33</td>
<td>60</td>
<td>18</td>
<td>7</td>
<td>21.48</td>
</tr>
<tr>
<td>34</td>
<td>60</td>
<td>18</td>
<td>7</td>
<td>23.08</td>
</tr>
</tbody>
</table>

Abbreviations: Exp No, Experiment numbers; T, reaction temperature (°C); t, incubation time (h); Mr, substrate molar ratio (mol/mol).
Table 5.2: ANOVA table for incorporation of palmitic acid

<table>
<thead>
<tr>
<th>Mol% C16:0 at sn-2</th>
<th>DF</th>
<th>SS</th>
<th>MS (Variance)</th>
<th>F</th>
<th>p</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>34</td>
<td>16580</td>
<td>487.648</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1</td>
<td>12956</td>
<td>12956</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected</td>
<td>33</td>
<td>3624.02</td>
<td>109.819</td>
<td>10.4794</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>9</td>
<td>3063.39</td>
<td>340.377</td>
<td>14.5714</td>
<td>0.0000</td>
<td>18.4493</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>560.622</td>
<td>23.3593</td>
<td>4.83314</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 34  DF = 24  $Q^2 = 0.673$  $R^2 = 0.845$  $R^2$ Adj. = 0.787

DF: Degrees of freedom; SS: sum of squares; MS: mean squares; F: F-value; p: p-value; SD: standard deviation; N: Number of observations. $Q^2$, $R^2$, and $R^2$ Adj, explained in text.
Table 5.3: Regression coefficients and significance (P-value) of the second-order polynomials for palmitic acid incorporation

<table>
<thead>
<tr>
<th>Mol% C16:0 at sn-2</th>
<th>Coef</th>
<th>Std err</th>
<th>P-value</th>
<th>Conf Int (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>22.5796</td>
<td>1.96929</td>
<td>3.19E-11</td>
<td>4.06437</td>
</tr>
<tr>
<td>T</td>
<td>-3.55481</td>
<td>0.924734</td>
<td>0.000780492</td>
<td>1.90853</td>
</tr>
<tr>
<td>t</td>
<td>-0.218456</td>
<td>0.924735</td>
<td>0.815253</td>
<td>1.90854</td>
</tr>
<tr>
<td>Mr</td>
<td>-7.31298</td>
<td>0.924735</td>
<td>3.87E-08</td>
<td>1.90854</td>
</tr>
<tr>
<td>T*T</td>
<td>-3.90844</td>
<td>1.01769</td>
<td>0.000787721</td>
<td>2.10039</td>
</tr>
<tr>
<td>t*t</td>
<td>-3.39591</td>
<td>1.0177</td>
<td>0.00275242</td>
<td>2.10039</td>
</tr>
<tr>
<td>Mr*Mr</td>
<td>3.49712</td>
<td>1.0177</td>
<td>0.00215602</td>
<td>2.10039</td>
</tr>
<tr>
<td>T*t</td>
<td>-1.26844</td>
<td>1.20829</td>
<td>0.304274</td>
<td>2.49375</td>
</tr>
<tr>
<td>T*Mr</td>
<td>2.01656</td>
<td>1.20829</td>
<td>0.108123</td>
<td>2.49375</td>
</tr>
<tr>
<td>t*Mr</td>
<td>0.314689</td>
<td>1.20829</td>
<td>0.796744</td>
<td>2.49375</td>
</tr>
</tbody>
</table>

*aAbbreviations: Coef, multiple regression coefficients; Std err, standard error; Conf int, confidence interval; T, temperature; t, time; Mr, substrate molar ratio; t, time; T*T, quadratic term of T; t*t, quadratic term of t; Mr*Mr, quadratic term of Mr; T*t, interaction term of T and t; T*Mr, interaction term of T and Mr; t*Mr, interaction term of t and Mr.*

*bCoefficients with P-value less than 0.05 are significant.
Table 5.4: Optimal conditions generated by Modde 5.0 (Umetrics, Umea, Sweden) software

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Temperature</th>
<th>Time</th>
<th>Substrate Ratio</th>
<th>Mol% C16:0 at sn-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>24</td>
<td>1.4</td>
<td>48.9</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>14.4</td>
<td>3</td>
<td>39.3</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>12</td>
<td>1</td>
<td>38.4</td>
</tr>
<tr>
<td>4</td>
<td>58.4</td>
<td>19.6</td>
<td>2.8</td>
<td>40.9</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>18</td>
<td>2</td>
<td>44.5</td>
</tr>
<tr>
<td>6</td>
<td>55</td>
<td>12</td>
<td>2</td>
<td>43.6</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>24</td>
<td>2</td>
<td>44.6</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>12</td>
<td>2</td>
<td>43.6</td>
</tr>
</tbody>
</table>

*aSet parameter ranges: substrate molar ratio (1-3 mol/mol), reaction temperature (55-65°C), reaction time (12-24 h).

Set target: 40% palmitic acid.
Table 5.5: Model verification using the $\chi^2$ (chi-square) test\textsuperscript{a}

<table>
<thead>
<tr>
<th>Region\textsuperscript{b}</th>
<th>Temperature (°C)</th>
<th>Substrate molar ratio</th>
<th>Time (h)</th>
<th>E</th>
<th>O</th>
<th>(O-E)$^2$/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>65</td>
<td>4</td>
<td>24</td>
<td>18.71</td>
<td>12.46</td>
<td>2.09</td>
</tr>
<tr>
<td>R2</td>
<td>60</td>
<td>1.1</td>
<td>18</td>
<td>50.49</td>
<td>52.97</td>
<td>0.12</td>
</tr>
<tr>
<td>R3</td>
<td>55</td>
<td>4</td>
<td>12</td>
<td>30.92</td>
<td>28.08</td>
<td>0.26</td>
</tr>
<tr>
<td>R4</td>
<td>52</td>
<td>7</td>
<td>18</td>
<td>18.26</td>
<td>18.52</td>
<td>0.0037</td>
</tr>
<tr>
<td>R5</td>
<td>60</td>
<td>7</td>
<td>18</td>
<td>22.58</td>
<td>20.71</td>
<td>0.15</td>
</tr>
</tbody>
</table>

$\chi^2 = \Sigma [(O-E)^2/E]$; E, expected incorporation (mol %); O, observed incorporation (mol %).

\textsuperscript{a}Response surfaces regions /Regions (R1-R5) with corresponding temperature, substrate ratio, and time.
Table 5.6: Fatty acid composition (mol%) and sn-2 fatty acid profile (mol%) of human milk and the infant formula fat analog produced under optimal conditions<sup>a</sup>

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fatty acid composition (Mol %)</th>
<th>Fatty acid at sn-2 (Mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formula fat analog</td>
<td>Human milk*</td>
</tr>
<tr>
<td>C8:0</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>C10:0</td>
<td>2.9</td>
<td>2</td>
</tr>
<tr>
<td>C12:0</td>
<td>23.6</td>
<td>7</td>
</tr>
<tr>
<td>C14:0</td>
<td>8.2</td>
<td>8</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.6</td>
<td>23</td>
</tr>
<tr>
<td>C18:0</td>
<td>6.2</td>
<td>7</td>
</tr>
<tr>
<td>C18:1</td>
<td>29.6</td>
<td>38</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.4</td>
<td>9</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.1</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Substrate ratio (3 mol/mol), reaction temperature (55°C), reaction time (24 0.7h).

*Source: Jensen (1989).
Figure 5.1: Normal probability plot of residuals for incorporation of palmitic acid.

Numbers inside the graph represents experimental values. The near linear distribution of the experimental values indicates a good model. Sample number (n) = 6.
Figure 5.2: Plot of observed values vs. predicted values of the generated model. Numbers inside graph represents experimental values. The near linear distribution of the experimental values indicates a good model. Sample number (n) = 6.
Figure 5.3: Contour plots showing effect of reaction temperature (°C), substrate molar ratio, and incubation time (h) on palmitic acid incorporation at the sn-2 position. The numbers inside the contour plots indicate the level of palmitic acid incorporation at substrate molar ratio of 1 (Fig 3A), at substrate molar ratio of 2 (B), and at substrate molar ratio of 3 (C). Sample number (n) = 6.
CHAPTER 6
CHARACTERIZATION AND OXIDATIVE STABILITY OF STRUCTURED LIPID:
INFANT MILK FAT ANALOG

ABSTRACT

Structured lipids (SLs) for infant milk formulation were produced by enzymatic interesterification of tripalmitin with vegetable oil blends and fish oil. The SLs were characterized by fatty acid content and structure, melting profiles, oxidative stability index, free fatty acid (FFA) concentration, and tocopherol content. Oxidative stability was studied using accelerated methods by quantifying FFA, peroxides (peroxide value) and aldehydes (p-anisidine value) production. Total oxidation (TOTOX value) was calculated as 2 x (peroxide value) + (p-anisidine value). The structured lipids after purification by distillation had melting profiles, oxidative stability index, and initial FFA concentration that were similar to that of the starting oils, while the fatty acid composition and structure of the SLs were similar to that of human milk fat. Oxidative stability of the SLs was improved with tocopherol addition as antioxidants and was comparable to that of the vegetable oils and oil blends.

KEY WORDS: Infant milk fat analog, oil blends, oxidative stability, oxidative stability index, palmitic acid, structured lipids.

INTRODUCTION

Developing infant milk fat similar to human milk fat (HMF) is of great interest and challenge to food processors. In most vegetable oils used for infant milk fat production, the sn-1,3 positions of the TAGs are occupied mainly by saturated fatty acids, while in human milk these positions contain mainly unsaturated fatty acids [1]. The location of saturated fatty acids especially palmitic acid at the sn-2 position of triacylglycerols (TAGs) increases the efficiency of absorption of fatty acids from the
lumen and also decreases calcium loss in infants [2]. Unlike vegetable oils, HMF contains about 40-60% palmitic acid at the sn-2 position of the TAGs [3].

Structured lipids containing similar fatty acid structure as HMF can be produced by interesterification reactions using an sn-1,3-specific lipase that gives high selectivity and mimics the natural pathways of metabolic processes [4]. Preliminary studies revealed that enzymatic interesterification of tripalmitin with a vegetable oil blend containing coconut, safflower and soybean oils, using lipozyme RM IM as a biocatalyst, can be successful in the production of SL that mimic the fatty acid composition and structure of HMF. Safflower and soybean oils are two sources of polyunsaturated fatty acids that together with coconut oil, produces an acceptable combination of fat that can be employed in infant milk fat formulation [5]. Furthermore, HMF contains small amounts of long chain polyunsaturated fatty acids (LCPUFAs), and the fortification of infant milk with these LCPUFAs by addition of fish oil is becoming increasingly popular for additional health benefits [6].

However, the successful production of SLs for infant milk formulation can be impeded by their high susceptibility to oxidative deterioration [7, 8, 9]. SLs or infant milks produced with lipids containing unsaturated fatty acids can deteriorate during storage and produce off-flavors and odors characteristic of oxidation [8, 10]. Furthermore, the production process for SLs increases FFA concentration, which is also responsible for off-flavor development [10]. High concentration of FFA in infant milk induces a rancid and bitter taste that is unacceptable. Also, unsaturated fatty acids in infant milk fat can be oxidized into hydroperoxides (primary oxidation products), which can then be rapidly decomposed to secondary oxidation products such as alkanes, alkenes, aldehydes and ketones [10]. Oxidative deterioration and high FFA concentration
can alter the flavor and nutritional quality of SLs [9] and render infant milks containing these lipids potentially toxic and unacceptable to consumers [10]. Therefore, the aim of this research was to characterize and study the oxidative stability of SLs intended as infant milk fat analog.

MATERIALS AND METHODS

Materials

Coconut, safflower and soybean oils were purchased from local retail outlets (Athens, GA). Menhadden fish oil, tripalmitin and mixed tocopherols were purchased from Sigma Chemical Co. (St. Louis, MO). Benzene, ethanol, methanol, glacial-acetic acid, hexane, chloroform, iso-octane and other organic solvents and chemicals were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ) or Fisher Scientific (Norcross, GA). Lipid standards including heptadecanoic acid, methyl esters of caprylic, capric, lauric, myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, arachidonic, behenic, eicosapentaenoic, and docosahexaenoic acids were purchased from Sigma Chemical Co. (St. Louis, MO). Lipozyme RM IM, an immobilized sn-1,3-specific lipase from *Rhizomucor miehei*, was obtained from Novozymes A/S (Bagsvaerd, Denmark). Apha (α-) tocopherol and tocotrienol standards were obtained from Fluka (Sigma Aldrich, St. Louis, MO) and beta (β-), gamma (γ-), and delta (δ-) tocopherol standards were obtained from Sigma Chemical Co. (St. Louis, MO).

*Preparation of oil blends.* Two different oil blends were prepared by mixing a 2.5:1.1:0.8, v/v/v blend of coconut, safflower, and soybean oils, respectively (B1), and by mixing a 2.1:1.1:0.8:0.4, v/v/v/v blend of coconut, safflower, soybean and fish oils, respectively (BFO). Coconut oil was melted to a liquid at 40 ± 2 °C before blending to ensure uniform distribution of the oil mixture.
**Synthesis of structured lipids.** Structured lipids (SLs) were synthesized in a packed-bed bioreactor by enzymatic interesterification of a 1:3 molar ratio of tripalmitin to B1 and BFO to give S1 and SFO, respectively. Lipozyme RM IM (10% weight of total reactants) was used as a biocatalyst in the reaction. The bioreactor was set-up according to the process described by Xu et al. [11]. The reaction was carried out at a reaction temperature of 55 °C and incubation time of 14.4 h.

**Purification of synthesized structured lipids.** The synthesized SLs were purified by removal of free fatty acids (FFA) as described by Akoh and Moussata [8]. A UIC KDL-4 short-path distillation system (UIC Inc., Joliet, IL) equipped with a 0.04 m² heated evaporated surface and 500 mL 2-neck receivers, was used for the purification process. Heating oil temperature was at 185 °C, while water temperature was at 20 °C. Free fatty acids were removed under vacuum (< 1 mmHg) from the SLs at a flow rate of 100 mL/h. The purified SLs (S1 and SFO) were both divided into 2 equal portions, and vitamin E (mixed tocopherols) was added to one portion at 1mg/4.4g (227ppm) to give S1V and SFOV, respectively. The second portion was left as is and served as a control. All samples were then flushed with nitrogen and stored at –80°C until needed for analysis.

**Characterization of structured lipids**

**Fatty acid analysis.** The fatty acid composition of the oil blends and SLs were determined. 0.1 g each of B1, BFO, S1 and SFO was pipetted into a screw-cap reaction tube. A 20 μL of heptadecanoic acid (0.1mg/mL) was added to each tube as an internal standard. Thereafter, 3 mL of 6% HCl in methanol was added and the mixture was methylated by incubating at 75 °C for 2 h in a pre-heated oven. Upon methylation, the
fatty acid methyl esters (FAME) was twice extracted with 2 mL hexane and 1 mL 0.1M KCl, and centrifuged at 1000 rpm (400 x g) for 3 min. The upper (hexane) layer was decanted and combined for each sample, then passed through anhydrous sodium sulfate columns. The excess solvent was evaporated under nitrogen until about 1 mL aliquot was obtained.

**Gas chromatographic (GC) analysis of FAME.** Fatty acid profiles were quantified using Agilent 6890N gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector (FID). Helium was the carrier gas and the gas flow rate was 1.7 mL/min. The oven temperature was initially held at 80 °C for 3 min and then programmed to 215 °C for 10 min at a rate of 10 °C/min, then held isothermally for 20 min. The column used was a fused silica Heliflex capillary column (Alltech-AT-225: 30 mm × 0.25 mm × 0.25 μm film thickness; Deerfield, IL). The different amounts of FAME (mol%) were analyzed and integrated by an integrator (model G2070AA, Agilent Technologies, Palo Alto, CA) with reference to C17:0 as an internal standard.

**Sn-2 positional fatty acid analysis by pancreatic lipase.** Fatty acids at the sn-2 position were analyzed according to the method described by Sahin et al. [4]. A 20 mg pancreatic lipase, 1 mL Tris buffer (pH 8.0), 0.25 mL bile salts (0.05%) and 0.1 mL calcium chloride (2.2%) were added to a test tube (25×200 mm) containing 0.1 g fat sample extracted from each of the infant formula analogs as described above. The sample reaction mixture was incubated at 40 °C in a water bath for 3 min. Then 1 mL of 6 M HCl and 1 mL diethyl ether were added, and the tube was centrifuged. Diethyl ether layer was evaporated under nitrogen stream (N-EVAP Organamation model No. 111) to a final volume of about 200 μL, which was spotted onto a silica gel G TLC plate and developed in a TLC tank by using hexane: diethyl ether: acetic acid (50:50:1, v/v/v) as the
developing solvent. The plate was sprayed with 0.2% 2,7-dichlorofluorescein in methanol and bands visualized under UV light. The 2-monoolein standard (Sigma) was used to confirm the TLC separation of 2-monoacylglycerol (2-MAG) in the reaction products. The 2-MAG band was then scrapped-off into a screw-capped test tube, extracted twice with 1 mL of hexane, and fatty acid methyl esters were prepared as aforementioned, and then analyzed by GC to evaluate the enzymatic incorporation of fatty acids at the sn-2 position of the TAG.

**Oxidative stability index.** The oxidative stability index (OSI) of the vegetable oils, fish oil, oil blends, SLs with added tocopherols (SIV and SFOV), and SLs without added tocopherols (SI and SFO) was determined at 110 °C with an Oil Stability Instrument (Ominion, Rockland, MA), according to the AOCS Cd 12b-92 method [12].

**Free fatty acids value.** The free fatty acids (FFA) value was determined by the AOCS Ca 5a-40 method [12]. Titration was done with KOH, using phenolphthalein as an indicator. The FFA was calculated as % oleic acid.

**Melting profiles determination.** The melting profiles of the samples were determined by differential scanning calorimetry according to the AOCS Cj 1-94 method [12].

**Tocopherol determination.** Tocopherol composition of the samples was determined according to the HPLC method described by Lee et al. [13], using a Shimadzu SRI203 HPLC system (Kyoto, Japan), equipped with a LC-6A pump, RF-10A spectrofluorometric detector. A normal phase column LiChrosorb Si 60 (5 μm 25.0 x 0.4 mm i.d.; Alltech Assoc., Inc., Deerfield, IL) coupled with a precolumn (30-40 μm) packed with Perisorb A, was used for the separation. The mobile phase consisted of hexane and 2-propanol (99.7:03, v/v). The excitation and emission wavelengths of the
detector were set at 290 and 330 nm, respectively. Five standard isomers (α, β, γ, δ, -tocopherol and α-tocotrienol) were used for the quantification of vitamin E in all samples.

Oxidation experiments

Five grams each of the vegetable oils, fish oil, oil blends, SLs and SLs with added tocopherols was weighed into soda-glass tubes (25 mm x 200 mm) and oxidized uncovered for 72 h at 60 °C in the dark using a shaking water bath (New Brunswick Scientific Co., Edison, NJ) as described by Akoh and Moussata [8]. The oils were sampled at 0, 24, 48, and 72 h of oxidation for FFA value, peroxide value (PV), and p-anisidine value (P-AV). PV was determined by the acetic acid – iso-octane method of AOCS Cd 8b-90 [12]. P-AV was determined by the spectrophotometric method of AOCS Cd 18-90 [12]. TOTOX (total oxidation) value was calculated as 2 x (PV) + (P-AV) as described by Shahidi and Wanasundra [14].

Statistical analysis

All experimental data were analyzed for analysis of variance, and mean differences between treatments (oils, oil blends, non-structured and structured lipids) using the general linear model program of SAS package [15]. All results are presented as means of replicates.

RESULTS AND DISCUSSION

Characterization of structured lipids

Fatty acid composition. The fatty acid composition of the oil blends and their corresponding SLs are given in Table 6.1. The enzymatic interesterification reaction increased the C16:0 content of the SLs. There was a 78% and 64% increase in this fatty acid content for S1 and SFO, respectively. The SLs showed a decrease in C8:0 and C10:0 content, in comparison to SFO, while the opposite trend occurred for C12:0 with about
6% greater decrease in SFO than S1 (Table 6.1). These decreases in medium chain saturated fatty acid content in the SLs can be attributed to the increased amount of C16:0 incorporated into the TAGs of the oils at the expense of the other saturated fatty acids (Table 6.1). The SLs have a fatty acid profile better simulated to the human milk fatty acid composition in comparison to the vegetable oil blends.

BFO contained LCPUFAs as a result of its fish oil content, which is also responsible for its significant EPA and DHA content compared to B1. Likewise, the LCPUFA content of SFO is attributable to the fish oil content of its starting oil blend (Table 6.1). Fatty acid composition of BFO and SFO indicate that the enzymatic interesterification process had minimal effect on the EPA and DHA concentration. About 94% and 92% of these LCPUFAs were retained after the interesterification reaction with sn 1,3 specific lipase from *Rhizomucor miehei*. These results are similar to those reported by Jennings and Akoh [7], Akoh and Moussata [8], and Sahin et al. [6].

**Sn-2 positional fatty acid composition.** The sn-2 positional fatty acid composition of the SLs and oil blends are given in Table 6.2. The incorporation of C16:0 at the sn-2 position increased significantly from the oil blends to the SLs due to the interesterification reaction. Both SLs (S1 and SFO) had about a 40-46% C16:0 incorporation, which suggests that most of the C16:0 contained in these SLs are located at the sn-2 position. This is unlike the results obtained for the unreacted oil blends (Table 6.2). Similar results were obtained by Nielsen et al. [10], where a good portion of the C16:0 was incorporated at the sn-2 position of the human milk fat substitutes produced by enzymatic reactions.

There was a decrease in the C12:0 at the sn-2 position of the SLs in comparison to their unreacted counterparts, which suggests that C16:0 was incorporated in this position
at the expense of C12:0. Trace amounts of EPA and DHA were also incorporated at the sn-2 position of SFO, which is due to the fish oil content of its starting material (Table 6.2). These results are similar to that reported by Sahin et al. [6], where less than 1% total EPA and DHA were incorporated at the sn-2 position after esterification reactions using lipozyme RM IM.

The SLs had increased amounts of C18:0 located at the sn-2 position, unlike the oil blends. This suggests that a good portion of this fatty acid migrated to the sn-2 position during the enzymatic interesterification reaction (Table 6.2). The amount of C18:0 at the sn-2 position of the SLs therefore appeared closer to that of HMF, in comparison to the oil blends, which show trace amounts of C18:0 at the sn-2 position. The sn-2 fatty acid profiles of the SLs more closely resemble HMF, in comparison to the oil blends.

Oxidative stability index. The OSI of the vegetable oils, fish oil, oil blends, SLs with added tocopherols and their controls are given in Table 6.3. Coconut oil had the highest oxidative stability in comparison to the other oils. This is due to the high content of saturated fatty acids in coconut oil, leading to a high resistance to oxidation. On the other hand, fish oil had the lowest OSI value (0.2 h at 110 °C), and therefore the lowest resistance to oxidative deterioration. The low OSI value of fish oil can be attributed to its high unsaturated fatty acids content especially the LCPUFAs. Although safflower and soybean oils, which contain high contents of monounsaturated fatty acids (MUFA) had lower OSI values than coconut oil, they also had better oxidative stability than fish oil (Table 6.3).

The oil blends have better stability to oxidation in comparison to safflower, soybean and fish oils (Table 6.3), with B1 showing a 64% higher OSI value at 110 °C.
than BFO. Increased saturated fatty acids content due to the addition of coconut oil to these oil blends could be the reason for their higher oxidative stability in comparison to the aforementioned vegetable oils. However, the higher OSI value of B1 in comparison to BFO can be attributed to the higher LCPUFA content of BFO. This higher LCPUFA in BFO can be traced to the presence of fish oil in this sample, and is also responsible for the increased susceptibility of BFO to oxidation, in comparison to B1. On the other hand, the enzymatic interesterification reaction appeared to increase the susceptibility of the SLs to oxidation, as the OSI values of S1 and SFO decreased significantly from that of B1 and BFO (Table 6.3). Factors such as unsaturated fatty acid composition and loss of tocopherols and phospholipids [8, 16] during the enzymatic reaction and short-path distillation process might be associated with the low stability of S1 and SFO samples. However, the addition of tocopherols as an antioxidant to the SLs positively affected their oxidative stability. The OSI values of SIV and SFOV were significantly higher than those of S1 and SFO (Table 6.3). Although antioxidant addition increased the oxidative stability of the SLs (Table 6.3), the presence of LCPUFAs also had an impact on the OSI values of these SLs, irrespective of their tocopherol content. Therefore the lower OSI value of SFO and SFOV at 110 °C relative to S1 and S1V (Table 6.3) can be attributed to higher LCPUFA content of the former in comparison to the latter. The results in Table 6.3 show that the OSI values of SIV and SFOV at 110 °C are more comparable to those of B1 and BFO, unlike the values of S1 and SFO at the same OSI temperature.

Free fatty acid content. The free fatty acid (FFA) content of the vegetable oils, fish oil and oil blends are given in Table 6.3. Although coconut oil had the least FFA content, the vegetable oils and oil blends all had FFA concentrations <1% (Table 6.3). The FFA concentrations in the SLs were comparable to those of the starting materials,
indicating that the short-path distillation process was successful in removing most of the FFAs generated during the enzymatic interesterification reaction. Akoh and Moussata [8] and Nielsen et al. [10] reported similar results of FFA removal using short-path distillation.

**Melting profile.** The melting profiles of the samples are given in Table 6.3. The vegetable oils have higher melting profiles than fish oil, which has a melting point range below 0 °C. Coconut oil has a melting profile similar to that of the vegetable oils, although coconut oil becomes liquid at temperatures above 24 °C. This melting range for coconut oil may be due to its high short-chain fatty acid content, which have low melting points. Unlike coconut oil, which solidifies at room temperature, safflower and soybean oils have melting points of –17 °C and –20 °C, respectively, and are liquids at room temperature. The oil blends on the other hand showed different melting profiles relative to their individual oil content. BFO had a slightly lower and narrower melting point range than B1 (Table 6.3), which might be due to its fish oil content. However, these oil blends appeared to have a narrower melting profile than the individual oils, which may be attributable to higher content of medium-chain saturated fatty acids, with closer melting points. The SLs on the other hand had higher contents of long-chain saturated fatty acids (C14:0 and C16:0) in comparison to the individual oils, and higher contents of C16:0 in comparison to the oil blends. The SLs had higher and wider melting profiles than the other oils (Table 6.3), attributable to the high degree of saturation of these oils relative to the individual oils and oil blends, and to the interesterification process. Tocopherol addition appeared to have minimal effect on the melting profiles of the SLs, because the melting point range of S1 and SFO were comparable to those of SIV and SFOV (Table 6.3).
Tocopherol content. The tocopherols content of the samples are given in Table 6.4. Safflower and soybean oil had the highest total tocopherol values, with soybean oil having about 67% higher tocopherols than safflower oil. Both of these oils appear to be lacking in α-tocotrienol. Tocopherol content of safflower oil consists mostly of α-tocopherol, while that of soybean oil consists of γ- and δ-tocopherols. The tocopherol content of coconut oil, though lower than that of the other vegetable oil blends, had the highest content of α-tocotrienol. The oil blends contained significant amounts of all 4 types of tocopherols (α, β, γ, δ) and α-tocotrienol, with α- and γ-tocopherol making the highest contribution to the total. β-Tocopherol was low in all samples relative to the other tocopherol homologues (Table 6.4). Fish oil had the lowest total tocopherol content (Table 6.4) consisting mainly of α-tocopherol and small amounts of δ-tocopherol. Jennings and Akoh [7] reported similar tocopherol results for unmodified menhaden fish oil.

Purification of the SLs by short-path distillation had a negative impact on the tocopherol content (Table 6.4). There was a 54% loss in total tocopherol content from the oil blends during the enzymatic reaction to produce the corresponding SLs and short-path distillation processes (Table 6.4). Akoh and Moussata [8] reported similar tocopherol losses, where enzyme processing and exposure to light and heat may be responsible for the losses. According to Hamam and Shahidi [17], the formation of tocoferyl esters during the interesterification reaction might also be responsible for the loss of endogenous tocopherols present in the oils. There were higher losses of α-tocopherol in the samples relative to other tocopherol types, which might be as a result of its higher heat lability and therefore greater susceptibility to destruction during the short-path distillation process. The addition of mixed tocopherols (up to 227 ppm) as antioxidants to
the SLs increased their total tocopherol content by about 42-50%, which is apparent in the total tocopherol content of S1V and SFOV, with respect to S1 and SFO.

**Oxidative stability of oils**

*Free fatty acid value.* The FFA concentrations for all samples during the accelerated oxidation period are given in Figure 6.1. The FFA values were low (<1%) for all samples throughout the 72 h accelerated oxidation period at 60 °C. There was no significant difference in the FFA content of safflower and soybean oils during the oxidation period (Figure 6.1). Coconut oil had the lowest FFA value from 0 – 48 h of accelerated oxidation, while its FFA value at 72 h was similar to those of the other vegetable oils (Figure 6.1). Although fish oil had the highest FFA content relative to the other individual oils (Figure 6.1), its FFA concentration at 72 h of accelerated oxidation was less than 0.6%, with a 40% total increase in this index from 0 - 72 h. This is significantly lower than that of coconut, safflower, and soybean oils with 75, 52, and 51% total increases, respectively (Figure 6.1). This low increase in FFA concentration in fish oil can be as a result of a higher rate of oxidation of the FFA produced to form primary oxidation products. Also, fish oil contains lower antioxidants and higher LCPUFAs than the vegetable oils. The oil blends on the other hand had FFA values that are comparable to that of the vegetable oils, although the initial value for BFO was slightly higher than that of B1 and the vegetable oils, which is attributable to its fish oil content. The SLs had higher FFA values than their starting materials, with SFO and SFOV having higher values than their counterparts due to their fish oil content. However, the higher initial FFA values (>0.1%) of the SLs in comparison to the vegetable oils and oil blends may have been as a result of increased FFA production during the enzymatic
interesterification process. The short-path distillation step resulted in a 0.3 - 0.4% residual FFA concentration in these SLs. Similar residual values were reported by Nielsen et al. [10], where about 0.3% FFA remained after the distillation process.

**Peroxide value.** The peroxide values (PV) of the oils are presented in Figure 6.2. Coconut oil had the lowest initial (< 0.1meq/1000g) and final (<0.2 meq/1000 g) values (Figure 6.2). Coconut oil also had the lowest increase in PV throughout the accelerated oxidation period, which is due to its lower unsaturated fatty acid content in contrast to the other oils. Fish oil had the highest increase in PV probably as a result of high LCPUFA content and low antioxidant content (Figure 6.2).

The SLs had lower initial PV in comparison to the oil blends. This may be as a result of the enzyme reaction and distillation processes, which increased the saturation and decreased the FFA and other volatile contents of the SLs. Akoh and Moussata [8] reported the stabilization of SLs to oxidative deterioration by incorporation of saturated medium chain fatty acid (caprylic). SFO had higher PV than S1 (Figure 6.2) throughout the accelerated oxidation period, likely because of its higher LCPUFA content in comparison to S1. Comparison of SFO to SFOV shows significantly lower values for the SL with added tocopherol. Similar result was obtained when S1 and S1V were compared, whereby S1V has lower PV than S1 (Figure 6.2).

The PV of S1V and SFOV in comparison to that of S1 and SFO shows the effect of tocopherol addition on the oxidative stability of the SLs. The PV appears to be quite similar for the SLs with added tocopherols, irrespective of their LCPUFA content.

**P-anisidine value.** P-anisidine assay is used to quantify the carbonyl compounds present in oils as a means to determine the past history of the oil [9, 14]. The vegetable oils had very low P-AV (Figure 6.3), unlike fish oil, which had an initial P-AV greater
than 1.0/g, and about 95% increase in this index after 72 h oxidation at 60 °C. The oil blends both showed a P-AV less than 0.2 after 72 h of oxidation (Figure 6.3). The high P-AV of fish oils can be attributed to a high rate of secondary oxidation products formation, unlike in the vegetable oils and oil blends.

Tocopherol addition as an antioxidant had a positive effect on the P-AV of the SLs throughout the accelerated oxidation period. Comparison of S1V and SFOV to B1, BFO, S1, and SFO shows that the tocopherol-fortified oils had lower P-AV (Figure 6.3). This is expected due to the low formation of hydroperoxides in these fortified SLs. Since the peroxides have to be present before subsequent breakdown to secondary oxidation products, it follows that low hydroperoxide formation increased the oxidative stability of these oils. The SLs with added tocopherols had P-AV that are comparable to their respective starting oil blends. S1 and SFO on the other hand, had P-AV at 24 h that are comparable to P-AV values of the oil blends at 72 h of accelerated oxidation (Figure 6.3). Fish oil, S1 and SFO had higher P-AV than other samples, which suggests low oxidative stability of these oils with respect to the other oils.

**TOTOX value.** The TOTOX values of the samples are given in Figure 6.4. Initial TOTOX values for all samples ranged from 0.1 to 10%, with coconut oil having the lowest value and fish oil the highest value (Figure 6.4). These results are expected since the TOTOX value is a combination of the PV and P-AV normally used to determine the total oxidative stability of the oil. The SLs had higher TOTOX values than the oil blends, which is also expected due to the low antioxidant content of these oils that make them less stable to oxidation. S1V and SFOV on the other hand had lower TOTOX values than those of the oils blends, as a result of the combined effect of tocopherols and stabilizing action of high saturated fatty acids content of these samples. The SLs with added
tocopherols had better total oxidative stability than their starting oils, and are therefore of better oil quality than the unfortified SLs.

REFERENCES


### Table 6.1: Fatty Acid Composition and sn-2 Positional Fatty Acid Profiles of the Oil Blends, Purified Structured Lipids, and Human Milk Fat

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>HM*</th>
<th>B1</th>
<th>BFO</th>
<th>S1</th>
<th>SFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>-</td>
<td>7.9±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1±0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C10:0</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.4±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.4±0.3&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>C12:0</td>
<td>7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.6±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.7±1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.6±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.1±1.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>C14:0</td>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.5±0.1&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>10.0±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2±0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.0±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:0</td>
<td>23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.2±0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.3±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.6±1.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.0±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.8±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>0.3±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:0</td>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.0±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.1±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.6±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.6±1.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.7±0.7&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.4±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.9±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.6±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1±0.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.5±0.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>1.3±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.2±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.3±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>0.2±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Source: [3, 6]; HM: Human milk fat; B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils; S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm); ND: Not detected. Data with the same letters within rows are not significantly different (p<0.05).
Table 6.2: Sn-2 Fatty Acid Profile of the Oil Blends, Purified Structured Lipids and Human Milk Fat

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>HM*</th>
<th>B1</th>
<th>BFO</th>
<th>S1</th>
<th>SFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>-</td>
<td>0.6±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.8±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.1±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C10:0</td>
<td>0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C12:0</td>
<td>2.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>67.5±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.9±1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.3±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.1±0.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C14:0</td>
<td>7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1±0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.0±0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.5±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1±0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:0</td>
<td>40-60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.8±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.9±1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>4.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Tr</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Tr</td>
<td>Tr</td>
<td>2.6±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.0±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.3±0.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.7±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>7.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.8±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6±0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.7±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.3±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>Tr</td>
<td>ND</td>
<td>0.1±0.0</td>
<td>ND</td>
<td>Tr</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>Tr</td>
<td>ND</td>
<td>0.1±0.0</td>
<td>ND</td>
<td>Tr</td>
</tr>
</tbody>
</table>

*Source: [3, 6]; HM: Human milk fat; B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils; S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm); ND: Not detected; Tr: Trace (0.05). Data with same letters within rows are not significantly different (p<0.05).
Table 6.3: Physico-Chemical Characteristic of the Vegetable Oils, Fish Oil, Oil Blends and Purified Structured Lipids

<table>
<thead>
<tr>
<th>Samples</th>
<th>Oxidative stability index at 110 °C (h)</th>
<th>Free fatty acid (%)</th>
<th>Melting profile (0 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil</td>
<td>79.1±0.8 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1±0.0 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>-35 to 27</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>3.5 ±0.3 &lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.1±0.0 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>-35 to 27</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.2±0.3 &lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.1±0.0 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>-34 to 26</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0.2±0.0 &lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.3±0.0 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>-31 to –6</td>
</tr>
<tr>
<td>B1</td>
<td>10.8±1.0 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2±0.0 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>-27 to 22</td>
</tr>
<tr>
<td>BFO</td>
<td>4.4±0.5 &lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.2±0.0 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>-28 to 22</td>
</tr>
<tr>
<td>S1</td>
<td>0.9±0.1 &lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.3±0.0 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>-27 to 38</td>
</tr>
<tr>
<td>SFO</td>
<td>0.9±0.2 &lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.4±0.0 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>-25 to 38</td>
</tr>
<tr>
<td>S1V</td>
<td>10.4±0.2 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3±0.0 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>-26 to 38</td>
</tr>
<tr>
<td>SFOV</td>
<td>2.7±0.5 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.3±0.0 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>-25 to 38</td>
</tr>
</tbody>
</table>

B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils

BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils

S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h

SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h

S1V: S1 with added tocopherols (227 ppm)

SFOV: SFO with added tocopherols (227 ppm)

Data with same letters within columns are not significantly different (p<0.05).
Table 6.4: Tocopherol Content (mg/100 g) of the Vegetable Oils, Fish Oil, Oil Blends and Purified Structured Lipids

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total tocopherol</th>
<th>α-tocopherol</th>
<th>β-tocopherol</th>
<th>γ-tocopherol</th>
<th>δ-tocopherol</th>
<th>α-tocotrienol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil</td>
<td>2.4±0.2h</td>
<td>0.19</td>
<td>0.0</td>
<td>0.2</td>
<td>ND</td>
<td>2.1</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>32.4±0.8b</td>
<td>30.4</td>
<td>0.5</td>
<td>1.2</td>
<td>0.3</td>
<td>ND</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>97.4±0.1a</td>
<td>6.6</td>
<td>0.6</td>
<td>60.3</td>
<td>29.9</td>
<td>ND</td>
</tr>
<tr>
<td>Fish oil</td>
<td>2.3±0.1h</td>
<td>2.3</td>
<td>ND</td>
<td>ND</td>
<td>0.0</td>
<td>ND</td>
</tr>
<tr>
<td>B1</td>
<td>27.2±1.7d</td>
<td>10.1</td>
<td>0.2</td>
<td>10.6</td>
<td>5.0</td>
<td>1.3</td>
</tr>
<tr>
<td>BFO</td>
<td>30.0±0.3c</td>
<td>11.5</td>
<td>0.3</td>
<td>11.6</td>
<td>5.4</td>
<td>1.2</td>
</tr>
<tr>
<td>S1</td>
<td>12.5±0.1f</td>
<td>2.2</td>
<td>0.0</td>
<td>6.2</td>
<td>4.1</td>
<td>ND</td>
</tr>
<tr>
<td>SF0</td>
<td>12.9±0.1f</td>
<td>2.9</td>
<td>0.2</td>
<td>6.3</td>
<td>3.2</td>
<td>0.3</td>
</tr>
<tr>
<td>S1V</td>
<td>24.4±0.9f</td>
<td>24.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td>SFOV</td>
<td>22.3±0.3f</td>
<td>21.1</td>
<td>0.0</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils
BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils
S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h
SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h
S1V: S1 with added tocopherols (227 ppm)
SFOV: SFO with added tocopherols (227 ppm)
ND: Not detected

Data with same letters within a column are not significantly different (p<0.05)
Figure 6.1: Free fatty acid concentration of vegetable oils, fish oil, oil blends, purified structured lipids control and structured lipids with added tocopherols, oxidized at 60 °C for 72 h. B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils; S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm). Sample number (n) = 6.
Free fatty acid value (%) vs. Time (h)

- Coconut oil
- Safflower oil
- Soybean oil
- Fish Oil
- B1
- BFO
- SI
- SFO
- S1V
- SFOV

The graph illustrates the change in free fatty acid value over time for different oils and blends.
Figure 6.2: Peroxide value of vegetable oils, fish oil, oil blends, purified structured lipids control and structured lipids with added tocopherols, oxidized at 60 °C for 72 h. B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils; S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm). Sample number (n) = 6.
Figure 6.3: P-anisidine value of vegetable oils, fish oil, oil blends, purified structured lipids control and structured lipids with added tocopherols, oxidized at 60 °C for 72 h.

B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils; S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm). Sample number (n) = 6.
Figure 6.4: TOTOX value of vegetable oils, fish oil, oil blends, purified structured lipids control and structured lipids with added tocopherols, oxidized at 60 °C for 72 h. B1: Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; BFO: Oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils; S1: Structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm). Sample number (n) = 6.
CHAPTER 7

PRODUCTION OF INFANT FORMULA ANALOGS BY MEMBRANE FRACTIONATION OF GOAT MILK: EVALUATION OF TEMPERATURE TREATMENT ON MEMBRANE PERFORMANCE

ABSTRACT

Goat milk was treated under different temperature conditions prior to a two-step-cascade membrane separation by ultrafiltration to eliminate β-lactoglobulin from the whey fraction. The effect of temperature treatment and membrane pore size on the elimination of β-lactoglobulin, and retention of α-lactalbumin was examined, and used to determine the optimum permeate fraction. The goat milk samples that were frozen at –35°C with and without prior heat treatment, showed the best membrane separation performance. The infant milk formula analogs produced using the casein and optimum permeate fractions of these milk preparations had the closest similarity to human milk with respect to concentrations of total protein (1.3 g/100g) and β-lactoglobulin (1-2 %), and casein-lactalbumin ratio (0.6-0.7). It was concluded that membrane performance during ultrafiltration of goat milk could be affected by temperature treatment of the milk prior to membrane separation.

KEY WORDS: Goat milk, Infant formula, ultrafiltration, membrane separation, β-lactoglobulin elimination.
INTRODUCTION

Goat milk has been recommended as a good substitute for cow milk and has become a more popular alternative base milk for the feeding of infants who cannot have breast-feeding, and for infants with cow milk allergies (Rosenblum and Rosenblum 1952; Park and Chukwu 1988; Park 1994, 2006). Goat milk is reportedly less allergenic and more digestible than cow milk (Taitz and Armitage 1984; Birkbeck 1978; Kirke 1979; Park 1994). Goat milk produces a softer and more friable curd than cow milk because goat milk is devoid of \( \alpha_{s1}\)-casein, a major protein in cow milk and therefore can be digested more readily and efficiently than cow milk (Jenness 1980; Haenlein and Caccese 1984; Chandan and others 1992).

Various authors reported that goat milk is different from cow or human milk in terms of higher digestibility, distinct alkalinity, higher buffering capacity, and therapeutic values (Gamble and others 1939; Rosenblum and Rosenblum 1952; Walker 1965; Devendra and Burns 1970; Haenlein and Cacces 1984; Park and Chukwu 1988; Park 1991). Virtually all infants who are allergic to cow milk may tolerate goat milk (Bahner and Heiner 1980; Park 1994). These infants may have been sensitive to cow lactalbumin, which is species specific, as well as to beta-lactoglobulin (\( \beta\)-Lg). \( \beta\)-Lg is a milk protein that is highly resistant to intestinal luminal hydrolysis and mostly responsible for milk allergy (Heyman and Desjoux 1992). This protein is a major whey protein in cow milk and is completely lacking in human milk (Park 1994; Park and Haenlein 2006). Goat milk contains lower \( \beta\)-Lg levels than cow milk, and milk of certain goat breeds and some species has little or no \( \beta\)-Lg content (Park 2006).

However, goat milk differs from human milk in both total protein content and casein/lactalbumin ratio. The protein energy ratio of whole goat milk is too high for its
use as an infant food, especially for the infants under one year of age (Parkash and Jenness 1968; Park 2006). The physical characteristics of the major goat milk proteins are shown in Table 7.1. Goat milk β-Lg, like its cow milk homolog, consists of a polypeptide chain of 162 amino acid residues (Jenness 1980; Park 2006), and has 3 less-negatively charged and one more positively charged residue than cow milk β-Lg at pH of 5 to 9. The removal of β-Lg from goat milk is difficult due to its pH dependency in the complex biological fluid system (Park 2006). Alpha-lactalbumin (α-La) on the other hand is a predominant whey protein in human milk and consists of about 30 % of the whey proteins in goat milk (Park 2006). Elimination of β-Lg from goat milk while retaining α-lactalbumin is, therefore, one step toward humanizing the protein composition of goat milk for infant feeding.

Most previous studies of milk fractionation have focused on salt/acid precipitation, ion exchange and affinity chromatography systems for selective protein purification. However, these systems have unacceptable economies at large scale (Zidney 1989; Pearce 1992), and require change in pH of the native milk system (Kuwata and others 1985), which might make the milk unacceptable for use in infant milk formulation. Membrane processes are used extensively throughout the dairy industry because they can be effectively and economically implemented at the large scale required for most dairy applications (Zydney 1998; Erdem and Yuksel 2005).

Temperature treatment is widely used to modify the properties of milk and milk products. The main change that occurs during temperature treatment of milk is denaturation of the milk proteins, which may cause a simultaneous change in permeate flux during subsequent membrane processing of the milk (Erdem and Yuksel 2005). No reports have been available on the modification of goat milk proteins by membrane
fractionation based on molecular size and temperature treatment, to eliminate $\beta$-Lg while retaining $\alpha$-La for infant feeding, at normal milk pH. Therefore, the objectives of this study were: (1) to lower the $\beta$-Lg and casein/whey protein ratio of goat milk without change in normal milk pH to simulate human milk protein composition, and (2) to determine the effect of temperature treatment on the separation performance of goat milk protein by ultrafiltration.

MATERIALS AND METHODS

Materials

Standard proteins of $\beta$-Lg and $\alpha$-lactalbumin were obtained from Sigma-Aldrich (St. Louis, MO). Water (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals used were of analytical and chromatographic grade. Polyethersulfone membrane flat sheets of 800, 30, 10, and 5 kDa molecular weight cut-off series were purchased from Synder filtrations, Inc. (Vacaville, CA). The membrane flat sheets were equilibrated with reverse-osmosis (RO) water and were operable in pH range of 1-14. Membranes were also compatible with both organic and aqueous media, and can be cleaned with RO water, nitric acid, alkali detergent (klenzade), and preserved in sodium metabisulfite.

Preparation of goat milk samples

Raw goat milk was obtained from the bulk tank milk of late lactation Saanen, Nubian, and Alpine dairy goat breeds at the Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, Georgia. The average daily milk yield was 0.5 L per head and the average somatic cell count of the milk was 550,000 per mL. The goats were fed Bermuda grass hay ad libitum, and 0.45 kg of concentrate
twice daily. The concentrate contained 5.0 % of crude fat and 2.72 % of digestible energy, which ensured 17 % of fiber in the total diet.

Two batches of raw goat milk were collected from the bulk tank at 4°C at Fort Valley, GA. One batch was treated as raw milk, and the other batch was immediately pasteurized at 63 °C for 30 min after collection. Both batches of the goat milk were divided into two equal parts. One part was immediately and continuously refrigerated at 4°C, and the other part was immediately frozen and stored at –35 °C, which gave the following temperature treated milks: RGM (raw, refrigerated goat milk); FGM (raw, frozen goat milk); PGM (pasteurized, refrigerated goat milk); and PFGM (pasteurized, frozen goat milk). Thereafter, the four temperature-treated goat milks and fresh goat milk as a control were tempered overnight at 4 °C, and then skimmed using a cream separator (ArmfieldFT15 Armfield, London) to separate milk fat from the skim milk.

*Ultrafiltration experiment*

Proteins in the temperature treated milks were separated using a batch ultrafiltration unit (NIRO Lab20, Hudson, WI), containing a fixed disc module (DOW, Denmark). The ultrafiltration unit was equipped with 76 mm radius flat disks, a diaphragm circulation pump and a shell and tube heat exchanger. Inlet pressure of the feed was maintained at 30 psi maximum, while outlet flow rate was maintained at 500 mL/min. The back-pressure valve was used to maintain the outlet flow rate.

Temperature treated milks were fractionated by ultrafiltration technique, in two steps using different membrane pore sizes (two-step-cascade) as illustrated in Figure 3.1. First-step fractionation was performed with the 800 and 30 kDa molecular weight cut-off membranes, using the flat disks of 76 mm actual radii and 56 mm effective radii. Milk samples were circulated through the ultrafiltration system until a 3x –retention factor was
obtained for all retentate. The retentate obtained from this first fractionation were respectively labeled as caseins, while permeates obtained were individually used as the feed for further fractionation. The second-step fractionation utilized 30, 10, and 5 kDa molecular weight cut-off membranes. The retentate and permeate milk fractions obtained were then labeled as illustrated in Figure 7.1. The skimmed fresh goat milk (SGM), and all fractions from the temperature treated milks were collected separately in flat pyrex dishes (200 mm x 150 mm x 20 mm) and frozen overnight at –40 °C. Thereafter, the samples were lyophilized using a freeze-drier (VirTis Freeze Mobile25, VirTis, Gardiner, NY) at a pressure of 133 x 10⁻⁴ kPa and temperature of –40±2 °C. The samples were then stored as lyophilisates in Styrofoam containers at –40±2 °C, until needed for further analysis.

**HPLC Analysis**

Analysis of the milk fraction samples was done by the HPLC method described by Kuwata and others (1985), using a Hewlett Packard (Series II 3247A) model 1090M equipped with a 1046A UV/VIS detector. Lyophilized samples were reconstituted as afore mentioned. Protein separation was carried out on a Bio-Sil SEC 125-5 column (300 x 7.8 mm) (Bio Rad Co., Richmond, CA) coupled with a Bio-Sil SEC 125 guard column (80 x 7.8 mm). The mobile phase was 0.05 M phosphate buffer (pH 6.0) containing 0.2 M sodium sulfate and 0.02 % sodium azide. The flow rate was 1 mL/min, and the effluents were monitored by measuring the absorbance at 225 nm or at 280 nm.

**Preparation of infant formula analogs**

The casein fraction of each milk sample was diluted to normal strength (3x dilution) with RO water. Optimum permeate fraction was chosen by determining the permeate with highest percentage β-Lg elimination and α-La retention for all samples.
Four different types of infant formulae were then prepared by combining one part of the diluted casein fraction and four parts of the corresponding optimum permeate fraction (v/v) obtained for each of the temperature treated milk. The prepared infant formula analogs were then lyophilized and stored as described above until needed for further analysis.

**pH measurement**

The pH of SGM, infant formula analogs, and milk fractions were determined using a pH-meter (Fisher Scientific, AR10, Fair Lawn, NJ). Prior to analysis, lyophilisates were reconstituted by mixing with RO water at the rate of (12.5:87.5 w/v).

**Total protein analysis**

Total protein content of the infant formula analogs was determined by total nitrogen assay using the Perkin Elmer 2400 Series II CHNS/O Analyzer. Total nitrogen content was multiplied by a factor of 6.25 to give the total protein content.

**RESULTS AND DISCUSSION**

**pH values**

The pH of the infant formula analogs, SGM, and milk fractions obtained from fractionation of the temperature treated goat milks are presented in Table 7.2. The range of pH for all samples was 6.2-6.5, which is closely simulated to the pH of human milk (6.3). All the milk fractions had similar pH values to their corresponding base milk and there was no significant (p < 0.05) difference in pH values amongst all milk fractions (Table 7.2). The infant formula analogs had pH values comparable to that of SGM and human milk. Temperature treatment and protein fraction therefore had no effect on the pH of the base milks, milk fractions and infant formula analogs.
**Comparison of temperature-treatment effect**

Temperature treatment had a great effect on the separation efficiency of the milk proteins. RGM showed a concentration of the major whey proteins (β-Lg and α-La) in the 800 kDa retentate fraction. About 70% of the total components in this fraction comprised of β-Lg and α-La, while these proteins were not detected in the rest of the fractions for this milk sample. This might be as a result of the pH dependency and ionic attraction of the natural goat milk proteins in the normal milk system, which enables an increase in their virtual size by surrounding ionic cloud (Zydney 1998), and renders their separation difficult with membrane molecular size of 30 kDa and below.

However, high temperature treatment of the milk prior to ultrafiltration separation of PGM appeared to give milk fractions with major whey protein contents comparable to that of RGM. Although heat treatment is known to cause denaturation of milk proteins to an extent, tempering of the milk at refrigeration temperatures also allows for refolding and renaturation of these milk proteins, whereby they tend to assume their normal form (Erdem and Yuksel 2005). Most of the major whey proteins in PGM are concentrated in the 800 kDa retentate fraction, just like in RGM, with very little or none of these proteins detected in the rest of the milk fractions.

FGM on the other hand showed a greater distribution of the major whey proteins in the 8000 kDa (34%), and 30 kDa (22%) retentate and 800/30 kDa (32%) permeate fractions, with about 87, 60 and 30% of the respective proteins attributable to β-Lg. This sample also showed the presence of major whey proteins in the 800/10, 800/5, 30/10 and 30/5 permeates. It, therefore, follows that the different behavior of the proteins in FGM during ultrafiltration in comparison with RGM, can be as a result of the frozen temperature treatment of FGM. This has probably resulted in a more fluid and flexible
structure of the protein molecules (Erdem and Yuksel 2005), thereby allowing permeation of these proteins through the 30 kDa membrane pores.

The combined high temperature and frozen temperature treatment of goat milk (PFGM) appeared to have a denaturation effect on the milk proteins, and the resulting behavior of these proteins during the subsequent ultrafiltration separation is comparable to that of FGM. The milk fractions of the PFGM sample had the highest major whey protein concentration in the 800 kDa (36 %), and 30 kDa (20 %) retentates, and 800/30 kDa (37 %) permeate. Although \( \beta \)-Lg accounted for about 86 and 57 % of the respective major whey protein values in the retentate fractions, its level in the permeate fraction was significantly lower (18 %), and was not detected in the other permeate fractions of this PFGM sample.

The four temperature treated samples showed the presence of residual major whey proteins in the casein fraction, which accounted for about 15-23 % of the total major whey proteins. This is expected since some of the whey proteins would always be present in the concentrated casein fraction, when using membrane separation techniques (Zydney 1998; Brans and others 2004). Higher levels (18-23 %) of these residual whey proteins were detected in RGM and PGM casein fractions than that of FGM and PFGM. These higher levels of residual major whey proteins in the caseins of the non-frozen milk samples may be as a result of the native whey protein dependency on the on the fluid milk system (Zydney 1998; Erdem and Yuksel 2005; Park 2006), which makes it more difficult to separate from the milk caseins. On the other hand, the residual whey proteins in FGM were comparable to that of PFGM. The lower residual whey protein content in the casein fraction of these samples can be as a result of relaxation of the natural rigidity
of the proteins caused by the heat and/or subsequent frozen temperature treatment of these samples (Erdem and Yuksel 2005).

Comparison of membrane performance

Elimination of β-Lg: The percentage elimination of β-Lg from the permeate fractions of the temperature treated milk samples are given in Figure 7.2. There appeared to be a complete elimination of the β-Lg in most of the permeate fractions of all four treated milk groups. Although the 800/30 kDa permeate fractions of FGM and PFGM groups showed a slight β-Lg content in comparison to that of other samples (Figure 7.2), no significant difference (p > 0.05) was observed in the percentage elimination of this protein amongst the permeates obtained from the different membrane sizes. The high efficiency of β-Lg elimination from the permeates of the second-step ultrafiltration fractionation of the four milk groups can be attributed to the ability of β-Lg to form a bipolymer in the absence of casein particles and presence of α-La, within the normal milk pH (Zydney 1998). This bipolymer formation of β-Lg in whey fractions can result in an increase in its molecular size, whereby its complete elimination occurred from the permeate fractions of membrane pore sizes of 10 kDa or less (Figure 7.2). A nearly complete elimination was also observed from that of 30 kDa membrane pore size (Figure 7.2). Furthermore, heat treatment at pasteurization temperatures prior to frozen temperature treatment appeared to have an effect on the efficiency of β-Lg elimination from milk whey. This is apparent from the higher (4 %) elimination of β-Lg in the 800/30 kDa permeate of PFGM in comparison with FGM (Figure 7.2). This might have been as a result of the frozen temperature treatment intensifying the degree of protein denaturation that occurred during pasteurization of the PFGM samples prior to membrane separation (Erdem and Yuksel 2005). Therefore, this situation caused a greater attraction of the
unfolded β-Lg molecules to form bipolymers (Zydney 1998), thus further preventing their passage through the second-step 30 kDa membrane pores.

**Retention of α-La:** The complete removal of β-Lg protein from goat milk whey by membrane filtration is highly desired for infant formula production. However, the retention of other whey proteins especially α-La is crucial to the determination of suitable membrane pore sizes for goat milk fractionation. The percentage retention of α-La in the different permeate fractions for the four milk types are presented in Figure 7.3. The 800/5, 30/10, and 30/5 kDa permeates of RGM and PGM showed no detectable α-La content (Figure 7.3). This inability of the α-La protein to permeate the pore sizes of 5 and 10 kDa membranes during the second-step fractionation of RGM and PGM can be attributable to the protein molecular size (14 kDa) in its natural state, which is bigger than the membrane pore size. The charged nature of the protein also enables it to attract electrons to itself, thereby resulting in an increase in its virtual molecular size (Zydney 1998; Tolkach and Kulozik 2005), and further prevent permeation through the membrane pores. The similarity of the α-La retention in the permeate fractions of PGM to RGM is likely due to the complete or partial refolding of this protein during refrigeration tempering to their natural form. These results are similar to that reported by Erdem and Yuksel (2005), where the whey proteins denatured to an extent by heat, returned to their original state during refrigeration storage.

The FGM and PFGM samples on the other hand showed a significant α-La retention in all permeate fractions (Figure 7.2). These higher levels of α-La distribution in the permeate fractions of FGM and PFGM unlike their non-frozen counterparts therefore signify that frozen temperature treatment of goat milk had a positive effect on the retention level of α-La in the different permeate fractions. The 800/30 kDa permeate
fraction of both FGM and PFGM milk samples contained the highest α-La level, which is up to 62 % higher than that of the other permeate fractions (Figure 7.2). This shows that the membrane pore size had a significant effect on the degree of α-La retention in the permeate fractions. These high α-La level in this fraction can be largely accounted for the molecular size of this protein (14kDa) which enables easy permeation through the 30 kDa membrane pore size and a restricted permeation through the 10 and 5 kDa membrane pore sizes.

**Protein composition**

The 800/30kDa-permeate was chosen as the optimum permeate fraction due to the higher level of β-Lg elimination and α-La retention obtained than in the other permeate fractions. This optimum whey permeate was therefore used for the preparation of the infant formula analogs.

The total protein contents of the infant formula analogs are compared to those of goat milk and human milk in Table 7.3. The infant formula analogs had lower protein contents than goat milk, due mainly to their reduced whey protein content as a result of whey protein reduction during the fractionation process, and the dilution of the caseins with the optimum whey permeate during preparation. RGM and PGM infant formula analogs had the lowest total protein content, while previously reported goat milk had the highest (Table 7.3). There appeared to be no significant difference (p > 0.05) between the protein content of RGM and PGM, and between that of FGM and PFGM. Human milk has a total protein content that is about 60 % lower than that of goat milk. Likewise, RGM and PGM infant formula analogs showed a lower protein content than the other formula analogs; with up to 50 % lower total protein in both infant formula analogs than in FGM and PFGM infant formula analogs (Table 7.3). The lower total protein content of
RGM and PGM infant formula analogs can be directly related to the lower whey protein content of their starting permeate material, which therefore reduced the total protein content of these samples. FGM and PFGM infant formula analogs on the other hand, showed a reduced total protein content that is lower than that of previously reported goat milk, but similar in content to human milk (Table 7.3).

The whey protein content of FGM and PFGM infant formula analogs also appeared higher (25 %) than that of documented goat milk, but closely similar to that of human milk (Table 7.3). RGM and PGM infant formula analogs had the lowest whey protein content, which is about 75 % lower than that of FGM and PFGM infant formula analogs (Table 7.3). The low whey protein content of both infant formula analogs can be attributed to the lower whey protein content of their starting material.

β-Lg protein accounts for about one-fifth of the total protein content of goat milk (Park and Haenlein 2006), but this protein is absent in human milk (Morgan 2006). The presence of β-Lg protein in the infant formula analogs (1-2 %) is as a result of the retention of his protein in the casein fraction of the milk samples. However, there appeared to be no significant difference (p > 0.05) in the β-Lg content of the infant formula analogs, though the PFGM analog had the lowest β-Lg content, followed by RGM (Table 7.3).

The ratio of casein to whey protein of the infant formula analogs is compared to that of the reported goat milk and human milk in Table 3. The casein-lactalbumin ratio of RGM and PGM appeared similar to that of the reported goat milk, while this index for FGM and PFGM is comparable to that of human milk (Table 7.3). The higher values of this index in RGM and PGM infant formula analogs are as a result of the low α-La content of these samples, unlike in FGM and PFGM. The results in Table 7.3 therefore
indicate that the FGM and PFGM infant formula analogs had the closest similarity to the protein composition human milk.

CONCLUSIONS

In this study, goat milk protein was fractionated by ultrafiltration to reduce the protein content and eliminate β-lactoglobulin. Temperature treatment of the milk can cause partial denaturation of milk proteins, which improves membrane performance during fractionation. The infant milk formula analogs produced had a similarity to human milk with respect to the total protein content, β-lactoglobulin content, and casein-lactalbumin ratio.

LITERATURE CITED


Table 7.1: Physical characteristics of the major whey proteins in goat milk

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration (g/100g)</th>
<th>Molecular weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-lactoglobulin</td>
<td>0.27</td>
<td>18,362</td>
</tr>
<tr>
<td>Alpha-lactalbumin</td>
<td>0.12</td>
<td>14,147</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>0.065</td>
<td>150,000-1,000,000</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.04</td>
<td>69,000</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.01</td>
<td>78,000</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>0.002</td>
<td>7,000</td>
</tr>
</tbody>
</table>

Data from Wong and others (1996); Cayot and Lorient (1997); Park (2006)
Table 7.2: pH of milk fractions and human milk

<table>
<thead>
<tr>
<th>Fractions</th>
<th>RGM</th>
<th>PGM</th>
<th>FGM</th>
<th>PFGM</th>
<th>HM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>6.51±0.14</td>
<td>6.51±0.12</td>
<td>6.51±0.13</td>
<td>6.51±0.12</td>
<td>6.25-6.29</td>
</tr>
<tr>
<td>Caseins</td>
<td>6.52±0.09</td>
<td>6.50±0.11</td>
<td>6.51±0.15</td>
<td>6.49±0.16</td>
<td>-</td>
</tr>
<tr>
<td>800kD-ret</td>
<td>6.28±0.03</td>
<td>6.29±0.16</td>
<td>6.28±0.12</td>
<td>6.30±0.14</td>
<td>-</td>
</tr>
<tr>
<td>800/30-per</td>
<td>6.29±0.04</td>
<td>6.29±0.11</td>
<td>6.30±0.13</td>
<td>6.29±0.09</td>
<td>-</td>
</tr>
<tr>
<td>800/10-per</td>
<td>6.27±0.12</td>
<td>6.28±0.03</td>
<td>6.27±0.02</td>
<td>6.27±0.11</td>
<td>-</td>
</tr>
<tr>
<td>800/5-per</td>
<td>6.27±0.04</td>
<td>6.27±0.13</td>
<td>6.28±0.04</td>
<td>6.27±0.12</td>
<td>-</td>
</tr>
<tr>
<td>30kD-ret</td>
<td>6.28±0.14</td>
<td>6.27±0.21</td>
<td>6.28±0.10</td>
<td>6.28±0.12</td>
<td>-</td>
</tr>
<tr>
<td>30/10-per</td>
<td>6.29±0.04</td>
<td>6.27±0.02</td>
<td>6.27±0.05</td>
<td>6.27±0.03</td>
<td>-</td>
</tr>
<tr>
<td>30/5-per</td>
<td>6.27±0.05</td>
<td>6.28±0.04</td>
<td>6.28±0.06</td>
<td>6.27±0.04</td>
<td>-</td>
</tr>
<tr>
<td>IFA</td>
<td>6.30±0.14</td>
<td>6.29±0.12</td>
<td>6.27±0.15</td>
<td>6.28±0.13</td>
<td>-</td>
</tr>
</tbody>
</table>

* Source: Jenness (1980).

HM: Human milk; 800kD-ret: 800kDa retentate fraction; 800/30-per: 800/30kDa permeate fraction; 800/10-per: 800/10kDa permeate fraction; 800/5-per: 800/5kDa permeate fraction; 30kD-ret: 30kDa retentate fraction; 30/10-per: 30/10kDa permeate fraction; 30/5-per: 30/5kDa permeate fraction; IFA: Infant formula analog; RGM: raw, refrigerated goat milk; FGM: raw, frozen goat milk; PGM: pasteurized, refrigerated goat milk; PFGM: Pasteurized, frozen goat milk.
Table 7.3: Comparison of protein composition of the infant formula analogs, goat milk and human milk.

<table>
<thead>
<tr>
<th></th>
<th>RGM</th>
<th>PGM</th>
<th>FGM</th>
<th>PFGM</th>
<th>GM**</th>
<th>HM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/100g)</td>
<td>0.74±0.23</td>
<td>0.71±0.14</td>
<td>1.52±0.13</td>
<td>1.41±0.12</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Whey protein (mg/100g)</td>
<td>0.22±0.03</td>
<td>0.14±0.04</td>
<td>0.92±0.10</td>
<td>0.81±0.12</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>β-Lg (%)</td>
<td>2.91±0.30</td>
<td>1.74±0.13</td>
<td>2.00±0.21</td>
<td>1.23±0.20</td>
<td>21.8</td>
<td>-</td>
</tr>
<tr>
<td>Casein: whey protein ratio</td>
<td>3.71±0.11</td>
<td>4.10±0.12</td>
<td>0.62±0.03</td>
<td>0.68±0.11</td>
<td>3.0-6.7</td>
<td>0.4-0.7</td>
</tr>
</tbody>
</table>

*Source: Jenness (1980); **Source: Park (2006)

RGM: Raw, refrigerated goat milk; FGM: Raw, frozen goat milk; PGM: pasteurized, refrigerated goat milk; PFGM: Pasteurized, frozen goat milk.
Figure 7.1: Schematic diagram of the membrane fractionation of goat milk samples by the two-step-cascade ultrafiltration technique. 800kD-ret: 800kDa retentate fraction; 800/30-per: 800/30kDa permeate fraction; 800/10-per: 800/10kDa permeate fraction; 800/5-per: 800/5kDa permeate fraction; 30kD-ret: 30kDa retentate fraction; 30/10-per: 30/10kDa permeate fraction; 30/5-per: 30/5kDa permeate fraction.
Skim milk

800 kDa membrane

30 kDa membrane

Casein

5 kDa membrane

10 kDa membrane

30 kDa membrane

5 kDa membrane

10 kDa membrane

800/5 kDa permeate

800/10 kDa permeate

800/30 kDa permeate

800 kDa retentate

800/10 kDa permeate

30/10 kDa permeate

30/5 kDa permeate

30 kDa retentate

Feed

Retentate

Permeate

First-step fractionation

Second-step fractionation
Figure 7.2: Percentage beta-lactoglobulin elimination in the permeate fractions. 800/30-per: 800/30kDa permeate fraction; 800/10-per: 800/10kDa permeate fraction; 800/5-per: 800/5kDa permeate fraction; 30/10-per: 30/10kDa permeate fraction; 30/5-per: 30/5kDa permeate fraction; RGM: Raw, refrigerated goat milk; FGM: Raw, frozen goat milk; PGM: pasteurized, refrigerated goat milk; PFGM: Pasteurized, frozen goat milk. Sample number (n) = 6.
Goat milk samples

Beta-lactoglobulin Elimination (%)

- 800/30
- 800/10
- 800/5
- 30/10
- 30/5
Figure 7.3: Percentage alpha-lactalbumin retention in the permeate fractions. 800/30-per: 800/30kDa permeate fraction; 800/10-per: 800/10kDa permeate fraction; 800/5-per: 800/5kDa permeate fraction; 30/10-per: 30/10kDa permeate fraction; 30/5-per: 30/5kDa permeate fraction; RGM: Raw, refrigerated goat milk; FGM: Raw, frozen goat milk; PGM: pasteurized, refrigerated goat milk; PFGM: Pasteurized, frozen goat milk. Sample number (n) = 6.
Figure 7.4: Percentage beta-lactoglobulin in the retentate fractions. 800kD-ret: 800kDa retentate fraction; 30kD-ret: 30kDa retentate fraction; RGM: Raw, refrigerated goat milk; FGM: Raw, frozen goat milk; PGM: pasteurized, refrigerated goat milk; PFGM: Pasteurized, frozen goat milk. Sample number (n) = 6.
Goat milk samples

Beta-lactoglobulin content (%)

- Casein
- 800kD-ret
- 30kD-ret

Goat milk samples
Goat milk can be simulated to human milk in terms of protein and fat composition. However, developing infant formula fat similar to human milk is of great interest and challenge to food processors. To simulate goat milk to human milk, the macronutrient content of goat milk has to be modified. This study has shown that ultrafiltration of goat milk proteins and blending of coconut, safflower, soybean, and fish oils can be used to develop milk formulas for infant food, which is important since unmodified goat milk is not suitable for infants in the first year of life.

In this study, goat milk protein was fractionated by ultrafiltration to reduce the protein content and eliminate beta-lactoglobulin. Temperature treatment of the milk can cause partial denaturation of milk proteins, which improves membrane performance during fractionation. The fat was structurally modified by enzymatic interesterification to simulate the triacylglycerol structure of human milk. Response surface methodology was used to model enzymatic experiments for optimal response, which can then be employed for up-scaling of the reaction. Optimal conditions were generated for substrate molar ratio, reaction temperature, and incubation time for production of the infant milk fat analog. The infant milk fat analog produced using the generated optimal conditions had total and sn-2 positional palmitic acid levels comparable to that of human milk, and were characterized by fatty acid content and structure, melting profiles, oxidative stability index, free fatty acid (FFA) concentration, and tocopherol content.
The infant milk formula analog produced by macronutrient modification of goat milk had a similarity to human milk with respect to the total protein content, β-lactoglobulin content, casein-lactalbumin ratio, total fat content, fatty acid profile, and acyl distribution on the glycerol backbone. The phytosterol and total sterol contents of the infant formula analog were also close to that of human milk. From the results of these studies, it can be concluded that the modified goat milk may be suitable for the development of infant milk fat and infant foods.

However, for the complete production of infant formula from goat milk, further studies would be necessary to simulate micronutrients (i.e., vitamins and minerals) of goat milk to human milk for infant feeding.