NUTRITIONAL VALUES AND STABILITY OF GOAT MILK TABLETS

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ABSTRACT

This study was carried out to assess the effect of plastic and aluminium laminated containers on the major nutritional composition, fat-soluble vitamins (A, E) and water-soluble vitamins (folic acid, B_1) of goat milk tablets. Pasteurized goat milk was spray dried. The milk powder obtained was used to prepare tablets by wet granulation method. The tablets were placed in plastic bottles and aluminium laminated pouches and stored at both room temperature and accelerated storage condition ($45\pm2\ ^0$C, 90% relative humidity). The major constituents of fat, protein, lactose and solid-not-fat, were analyzed by a MilkoScan device while vitamins A, E, B_1 and folic acid were determined by high performance liquid chromatography (HPLC). The initial contents of vitamins A and E were 0.0780 and 0.1000 µg/g, respectively. For folic acid and vitamin B_1, the initial contents were 0.0499 and 0.0779 µg/g, respectively. After 90 days of storage at room temperature and at the accelerated storage condition, the composition of the goat milk tablets (fat, protein, lactose and solid-not-fat) was unchanged in both types of containers. After storage at room temperature for 90 days, the content of vitamin E, vitamin B_1, and folic acid in goat milk tablets was unchanged in both containers while the content of vitamin A decreased slightly. This was not statistically significant in both plastic bottles and aluminium laminated pouches. At the accelerated condition, the content of vitamin A in the goat milk tablets decreased to 0.0606 µg/g (%loss = 22.34) and 0.0702 µg/g (%loss = 10.00) in the plastic bottles and aluminium laminated pouches, respectively. The content of vitamin E decreased by 12.15% and 4.22% in the plastic bottles and aluminium laminated pouches, respectively. The content of folic acid decreased by 74.89% and 51.98% while vitamin B_1 decreased by 57.54 and 41.33% in the plastic bottles and aluminium laminated pouches, respectively. The magnitude of change in terms of these nutritional parameters suggests that the effect of plastic bottles and aluminium laminated pouches is not significantly different ($P>0.05$).

KEYWORDS: GOAT MILK TABLET/FAT-SOLUBLE VITAMINS/WATER-SOLUBLE VITAMINS/ PLASTIC BOTTLE /ALUMINIUM LAMINATED POUCH

83 pages

คุณค่าทางโภชนาการและความคงตัวของนมแพะอัดเม็ด

NUTRITIONAL VALUES AND STABILITY OF GOAT MILK TABLETS

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บทคัดย่อ

การวิจัยนี้เป็นการศึกษาถึงผลของภาชนะบรรจุที่ใช้บรรจุนมแพะอัดเม็ดต่อปริมาณสารประกอบหลัก วิตามินที่ละลายในไขมัน (เอ, อี) และวิตามินที่ละลายในน้ำ (กรดโฟลิค, บีหนึ่ง) นมแพะอัดเม็ดได้จากการทำนมแพะพาสเจอไรซ์ให้เป็นผงด้วยเครื่อง Spray dryer และทำให้เป็นเม็ดด้วยวิธี wet granulation นมแพะอัดเม็ดที่ได้นำมาบรรจุในขวดพลาสติกและถุงอลูมิเนียมลามิเนท ก่อนที่จะนำไปเก็บที่สภาวะอุณหภูมิห้องและที่สภาวะเร่ง (45 องศาเซลเซียส ความชื้นสูง) และตรวจปริมาณสารประกอบหลักในนมแพะอัดเม็ด ได้แก่ ไขมัน โปรตีน แลคโตส และธาตุน้ำนมไม่รวมมันเนย โดยใช้เครื่อง MilkoScan การวิเคราะห์ปริมาณวิตามิน เอ อี วิตามินบีหนึ่งและกรดโฟลิค ใช้วิธีไฮเพอร์ฟอร์มานซ์ลิควิดโครมาโทกราฟี ปริมาณเริ่มต้นของวิตามิน เอ อี กรดโฟลิคและบีหนึ่งเท่ากับ 0.0780, 0.1000, 0.0499, และ 0.0779 ไมโครกรัมต่อกรัมตามลำดับ หลังการเก็บเป็นเวลา 90 วันทั้งในสภาวะอุณหภูมิห้องและที่สภาวะเร่ง ไม่พบการเปลี่ยนแปลงของสารประกอบหลักของนมแพะอัดเม็ดที่เก็บในภาชนะบรรจุทั้งสองชนิด สำหรับปริมาณวิตามินอี กรดโฟลิค และวิตามินบีหนึ่ง ไม่พบการเปลี่ยนแปลงหลังการเก็บเป็นเวลา 90 วันที่สภาวะอุณหภูมิห้อง ในขณะที่ปริมาณวิตามินเอลดลงจากเดิมเล็กน้อยซึ่งไม่พบการเปลี่ยนแปลงอย่างมีนัยสำคัญในทั้งขวดพลาสติกและถุงอลูมิเนียมลามิเนท ที่สภาวะเร่งปริมาณวิตามินเอลดลงจากเดิมร้อยละ 22.34 และ 10.00 ส่วนปริมาณวิตามินอีลดลงจากเดิมร้อยละ 12.15 และ 4.22 ในขวดพลาสติกและถุงอลูมิเนียมลามิเนท ตามลำดับ ปริมาณกรดโฟลิคลดลงจากเดิมร้อยละ 74.89 และ 51.98 ในขณะที่ปริมาณวิตามินบีหนึ่งลดลงจากเดิมร้อยละ 57.54 และ 41.33 ในขวดพลาสติกและถุงอลูมิเนียมลามิเนท ตามลำดับ จากการศึกษาผลของนมแพะอัดเม็ดที่บรรจุในขวดพลาสติกและถุงอลูมิเนียมลามิเนทต่อการเปลี่ยนแปลงของสารอาหารในนมแพะอัดเม็ดพบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ($P > 0.05$)

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LIST OF ABBREVIATIONS

AR	analytical grade
AOAC	The Association of Official analysis Chemist
A_w	Water Activity
cm	centimeter
^{O}C	degree celcius
g	gram
LOD	limit of detection
LOQ	limit of quantitation
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mM	millimolar
M	molar
nm	nanometer
r^2	correlation coefficient
rpm	round per minute
RSD	relative standard deviation
SD	standard deviation
t_R	retention time
UV	ultraviolet light
w/v	weight by volume
µg	microgram
µL	microliter
µm	micrometer

CHAPTER I
INTRODUCTION

In recent years, goat milk plays an important role in the nutrition of human diet, since it possesses unique properties, which distinguish it from cow milk and make it a valuable alternative, not just for infants but for the general population in the case of digestion problems. Goat milk can often be consumed by people who have allergies to cow milk. The nutritional advantage of goat milk compared to cow milk has been attributed to the small size of fat globules and probably this is one of the reasons for the easy digestion of goat milk (1). Goat milk has higher percentage of short and medium-chain (C_6-C_4) than cow milk, these fatty acids are considered interesting in medicine because they are used for the treatment of various aliments (e.g., malabsorption syndromes, intestinal disorders, coronary diseases and cystic fibrosis) (2). Goat milk has more calcium, phosphorous, potassium, magnesium, chloride and selenium, and less sodium and sulfur contents than cow milk. In addition, goat milk has higher amount of vitamins A and D than cow milk (3).

At present, the production of goat milk in Thailand is increasing but can not attract people to increase the consumption. Because of goat milk has a goaty odour which is not satisfying. Goat milk can be processed into different milk products, including goat fluid milk, powder, yogurt and cheese (4). In Thailand UHT and pasteurized goat milk and yogurt are available. However, goat milk powder is not yet available. Goat milk tablet can probably be an interesting alternative to produce because it is easy to eat and can bring to everywhere. Besides, the goaty odour can decrease by adding various flavouring agents.

There have been several reported about water-soluble and fat-soluble vitamins contents and their stability during storage in cow milk products both in liquid and in powder forms (5-9). However, there is no information on the stability of vitamins A, E, B_1 and folic acid in goat milk products.

The objectives of this work were to produce goat milk tablets and to study the effect of plastic bottles and aluminium laminated pouches containers on nutritional values and stability of goat milk tablets. The nutritional values in this study consist of major milk components (protein, fat, lactose and solid-not-fat), fat-soluble vitamins and water-soluble vitamins. The four major milk components, protein, fat, lactose and solid-not-fat (SNF), were analyzed by Milkoscan, an automatic milk analyzer. For vitamin components in milk, vitamins A and E were chosen to represent fat-soluble vitamins, and vitamin B_1 and folic acid (B_9) to represent water-soluble vitamins. The contents of vitamins were determined by high performance liquid chromatography.

CHAPTER II
LITERATURE REVIEW

2.1 Goat milk

2.1.1 Physico-chemical characteristics

Goat milk is white in colour compared to cow milk, which is yellowish because of the presence of carotene. Goat milk has stronger flavour than cow and sheep milk. This might be due to the liberation of short-chain fatty acid, which gives off a goaty smell. Unlike cow milk, which is slightly acidic, goat milk is alkaline in nature, which is very useful for people with acidity problems. This alkalinity is due to higher protein content and a different arrangement of phosphates (10). The compositions of goat, sheep, cow and human milk are given in Table 1 (11).

Table 1 Comparative compositions of milk from different species

Component	Goat	Sheep	Cow	Human
Fat (%)	3.8	7.9	3.6	4.0
Solid-not-fat (%)	8.9	12.0	9.0	8.9
Lactose (%)	4.1	4.9	4.7	6.9
Protein (%)	3.4	6.2	3.2	1.2
Casein (%)	2.4	4.2	2.6	0.4
Albumin, globulin (%)	0.6	1.0	0.6	0.7
Non-protein N (%)	0.4	0.8	0.2	0.5
Ash (%)	0.8	0.9	0.7	0.3
Calories/100ml	70	105	69	68

Compositions of goat, sheep, cow and human milk are different, due to variation in diet, breed, season feeding, environmental conditions, locality and health status of the udder (11). Goat milk contains higher fat and protein contents than cow milk. Overall, sheep milk contains higher total solids and major nutrient contents than goat and cow milk. Remeuf and Lenoir (11) reported that the relative proportions of the major casein components of goat milk are slightly different from those in cow milk. Goat milk contains less α_s-casein content and has more α_{s2} content than α_{s1}-casein content. Proportion of κ-casein and β-casein are higher in goat milk than in cow milk. The density of goat milk is equal to that of cow milk, but is lower than that of sheep milk. Surface tension and freezing point of goat milk is within the range of cow milk, but viscosity and acidity of goat milk is slightly higher. The physical properties of goat milk compared to sheep and cow milk are summarized in Table 2 (3).

Table 2 Some physical properties of goat, sheep and cow milk

Property	**Goat milk**	**Sheep milk**	**Cow milk**
Specific gravity (density)	1.029-1.039	1.0347-1.038	1.0231-1.0398
Viscosity, (C_p)	2.12	2.86-3.393	2.0
Surface tension (Dynes/cm)	52.0	44.91-48.70	42.3-52.1
Conductivity (Ω^{-1} cm^{-1})	0.0043-0.0139	0.0038	0.0040-0.0055
Refractive index	1.450±0.39	1.3492-1.3497	1.451±0.35
Freezing point (- ^{0}C)	0.540-0.573	0.570	0.530-0.570
Acidity (lactic acid %)	0.14-0.23	0.22-0.25	0.15-0.18
pH	6.50-6.80	6.51-6.85	6.65-6.71

2.1.2 Carbohydrates in goat milk

Milk sugar, lactose, is the major carbohydrate in goat milk. Lactose is important for maintaining osmotic equilibrium between the blood stream and the alveolar cells of the mammary gland during milk synthesis. Lactose is found in varying concentrations in the milk of all mammals except for seals. The lactose content of goat milk is about 0.2-0.5% less than that of cow milk (11).

2.1.3 Lipid in goat milk

Lipids are important components of milk in term of nutrition, physical and sensory characteristics that they impart to dairy products. In goat milk, lipid is present in the form of globules, which is characteristic abundant in sizes less than 3.5 μm. Some studies reported that the average fat globule size was smallest in sheep milk follow by goat milk (65% of globules less than 3 μm). This is an advantage for digestibility and more efficient lipid metabolism comparison to cow milk fat (12).

Short and medium chain fatty acids are significantly higher in goat milk than in cow milk (12). These fatty acids are considered to be of interest in medicine because they are used for the treatment of various aliments (e.g., malabsorption syndromes, intestinal disorders, coronary diseases and cystic fibrosis) (2).

2.1.4 Proteins in goat milk

The average protein content in goat milk (3.4%, w/w) is higher than in cow milk (3.2%, w/w). Protein contents vary within species, and influenced by breed, stage of lactation feeding, climate, season and udder health status. The principal protein in goat milk is about the same as in cow milk. Milk proteins occur in two distinct phases. One is an unstable micellar phase composed of caseins (α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein) and the other is a soluble phase composed of whey proteins (β-lactogloburin, α-lactalbumin and serum albumin) (13).

2.1.5 Vitamins and minerals.

Mineral contents of goat milk are much higher than those in human milk. Goat milk has more calcium, phosphorous, potassium, magnesium, chloride, zinc and selenium, and less sodium and sulfur contents than cow milk (Table 3). Whereas goat

and cow milk contain significantly greater iodine content than human milk, which would be important for human nutrition. Since iodine and thyroid hormones are involved in the metabolic rate of physicological body functions (14). In addition, goat milk has higher amount of vitamins A, D and C than cow milk (Table 3). Because goats convert all β-carotene into vitamin A in the milk, thus is the reason for it is whiter than cow milk.

Table 3 Mineral and vitamin contents (amount in 100 g) of goat and cow milk (11)

Constituent	**Goat milk**	**cow milk**
Mineral		
Ca (mg)	134	122
P (mg)	121	119
Mg (mg)	16	12
K (mg)	181	152
Na (mg)	41	58
Cl (mg)	150	100
S (mg)	28	32
Fe (mg)	0.07	0.08
Cu (mg)	0.05	0.06
Mn (mg)	0.032	0.02
Zn (mg)	0.56	0.53
I (mg)	0.022	0.021
Se (mg)	1.33	0.96
Vitamin		
Vitamin A (IU)	185	126
Vitamin D (IU)	2.3	2.0
Thiamine (mg)	0.068	0.045
Riboflavin (mg)	0.21	0.16
Niacin (mg)	0.27	0.08
Pantothenic acid (mg)	0.31	0.32
Vitamin B_6 (mg)	0.046	0.042
Folic acid (μg)	1.0	5.0
Biotin (μg)	1.5	2.0
Vitamin B_{12} (μg)	0.065	0.357
Vitamin C (mg)	1.29	0.94

2.2. Dry milk production

The advantages of dry milk over liquid milk are better keeping quality, less storage space and lower shipping costs (15). Milk powder is made by removing water from liquid milk. There are two methods, roller drying and spray drying, used for drying milk to powder form. Spray drying is the most important method of drying milk and milk products. By spraying in to a stream of hot air, the droplets formed present an extremely large amount of surface area and get dried immediately due to rapid evaporation of moisture. Milk is preheated and concentrated to 40-45% of total solids. Hot air is filtered and directed in to the drying chamber. The concentrated milk is atomized to obtain small particles ranging from 10-100 μm in diameter. The air leaving the drying chamber enters a cyclone separator where the fine particles are collected. The dried products are cooled, sifted and packed in suitable packaging material (16).

Omprakash developed a technology of drying goat milk which is similar to that of cow milk. Goat milk is standardized to 3.5% fat (solid not fat: fat, 2.7) and preheated to 90 °C. After that, it is concentrated to 32% total solids in a vacuum evaporator. Concentrated milk is homogenized in a two stages (stage 1 = 150 kg/cm^2; stage 2 = 40 kg/cm^2), then dried in a double drum drier at 160 °C, or in a spray drier with inlet and outlet air temperatures of 180 and 95 °C, respectively (17).

2.2.1 Milk tablets

Tablets are formed by compressing the mixture of active substance and excipients. In tablet processing, it is important that all ingredients be fairy dry, powder or granular, some what uniform in particle size, and freely flowing. All the ingredients should be well mixed. There are three methods for tablet production: direct compression, wet and dry granulation. The steps in these methods are shown in Table 4 (18).

Wet granulation method is widely used because this method ensures the content uniformity of the tablet. The advantage of this method is the use of compressibility less than other method and has a good content uniformity.

Table 4 Various methods for tablet production

Direct compression	Dry granulation	Wet granulation
Blend	Blend	Blend
Compress	Slug	Wet with binder
	Break down	Wet granulate
	Mix with lubricant	Dry
	Compress	Break down
		Mix with lubricant
		Compress

Moreover, wet granulation method can protect segregation of mixture between processing because the components of each granule are the same as mixture during binder solution adding. The properties of good tablets must be sufficiently strong and resist to handling during producing, packaging, transporting and use. Besides, tablets must be uniform in weight and active ingredient (18).

2.2.2 Packaging of product

Food packaging requires protection, tempering resistant, and special physical, chemical or biological needs. Besides, it shows nutrition information on the food being consumed. Roles of packaging are described below (19),

- Physical protection - The food enclosed in package may require protection from shock, vibration, compression, temperature, etc.
- Barrier protection- A barrier from oxygen, water vapor, dust, etc.,
- Containment or agglomeration - Small items are typically grouped together in one package for reasons of efficiency. Powder and granular materials need containment.
- Information transmission - Package and labels communicate how to use, transport, recycle, or dispose of the package.
- Marketing - The packaging and labels can be used by marketers to encourage potential buyers to purchase the product.

- Security – Packaging can play an important role in reducing the security risk of shipment and reduce the risk of package pilferage (19).

2.3 Change of nutritional factors in stored milk powder

2.3.1 Vitamins

The extent of vitamin losses during storage depends on the sensitivity of each vitamin. Vitamins may be affected by various factors such as the type of packaging and the length and conditions of storage (e.g., exposure to oxygen, light, and high temperature).

2.3.1.1 Fat-soluble vitamins

In general, vitamin A refers to all-*trans*-retinol, which is the most active form of this vitamin (20). Vitamin A seems to be very stable in a nitrogen atmosphere and in a dark cool place, but is readily oxidized with exposure to air and light (21). A number of previous studies have investigated the stability of vitamin A as affected by subsequent storage at various conditions. Albalá-Hurtado *et al.* (5) found significant losses (35.7%) of vitamin A during storage for 12 months at 37 ^{0}C but slightly decrease, which was not statistically significant, at 20 and 30 ^{0}C. Chávez-Servín *et al.* (22) reported that vitamin A content decreased (4.7-34.3%) when 20 commercial infant formulas were stored at 25 ^{0}C for 70 days. Chávez-Servín *et al.* (23) observed that vitamin A content reduced by 27-29% when two infant milk-based powder formulas were stored at 40 ^{0}C for 18 months. Vidal-Valverde *et al.* (24) reported that retinol loss 16% in milk powder stored at 20 ^{0}C and A_w 0.44 for 20 days.

Vitamin E is the main physiological fat-soluble antioxidant (25). The stability of vitamin E can be affected by environmental factors such as light, oxygen, temperature and moisture content (26). Several studies about the stability of vitamin E in milk have been reported. In powder and liquid infant milk formulas, Albalá-Hurtado *et al.* (5) found that the storage for 12 months at 37 ^{0}C did not affect the vitamin E content. On the other hand, Miquel *et al.* (26) observed that vitamin E content in four milk-based infant formulas kept 37 ^{0}C for 17 months decrease about 50%. Valverde *et al.*

(27) reported losses in α-tocopherol 17% in powder milk stored at 20 ^{0}C A_w 0.44 for 20 days and decrease significantly after 2 months of storage. Chávez-Servín *et al.* (22) observed that vitamin E content of 20 commercial infant formulas were stable when stored at 25 ^{0}C for 70 days. In the other hand, Chávez-Servín *et al.* (23) found that vitamin E content decreased by 23-28% when two infant milk-based powder formulas were stored at 40 ^{0}C for 18 months.

2.3.1.2 Water-soluble vitamins

Thiamine (vitamin B_1) is quite stable below pH 5.5. At above pH 7.0, it can be destroyed very fast even at room temperature. Folic acid (vitamin B_9) is easily destroyed in light and high temperature. There are a few studies about the effect of storage conditions and time on the content of milk powder. Albalá-Hurtado *et al.* (5) found that thiamine and folic acid content remained unchanged for 12 months when kept at 37 ^{0}C in powder and liquid infant milk formulas. Ford JE *et al.* (28) reported that the content of thiamine decreased about 18% and folic acid decreased substantially about 80% after storage at 60 ^{0}C for 8 weeks. Besides, there is a report for stability of vitamin B_1 in powder enteral formulas. Frias J *et al.* (29) found that the thiamine small decrease about 7-9% when stored at 30 ^{0}C for 3 months. Frias J *et al.* (21) reported that the thiamine content underwent a slight decreased (4-5%) when powder enteral formulas were stored at 30 ^{0}C with A_w 0.44 for one month.

2.3.2 Proteins and amino acids

In the case of skim milk powder, lysine and the sulfur-containing amino acids are destructed during the high temperature treatments of milks or Maillard reaction. The studies have shown that methionine and tryptophane content do not change significantly during storage at temperature ranging from -40 to 40 ^{0}C and water activities in the 0.15 to 0.41 range. The available lysine decreases most at high water activity and at the highest temperature. However, losses of lysine after 6 months of storage at 20 ^{0}C (at any water activity level) are less than 8%. It is only during extended storage (over 6 months) at temperatures exceeding 40 ^{0}C and at high water activity that the loss of lysine, and therefore the change of nutritional quality of milk

proteins, becomes more significant (with losses of 15-24%). Such extreme conditions are rarely encountered in commercial situations (30).

2.3.3 Lipids

The most significant deteriorative changes that occur in dry milk products that result in sensory, functional, and visual defects, are oxidation of the milk lipids. The oxidative stability of milk-fat in milk powder is influenced by the heat treatment of the milk, storage temperature, water activity and packaging (31).

2.4 Analytical method of milk

The studies of milk components on the quality of milk, the accuracy, precision, specificity, and linearity of an analytical method are very important. Chemical analysis and microbiological analysis are usually carried out on milk both before and after processing (32).

2.4.1 Major constituents

In recent years, there has been a growing trend towards instrumental methods, although in some cases the traditional method remains definitive. Infrared measurements, for example, are acceptable as a screening method for major components including solid-not-fat and total solid. This measurement can be categorized as two techniques. First, infrared milk analysis (IR) (33, 34) is based on absorption of IR energy at specific wavelengths by carbonyl groups in ester linkages of fat molecules (5.723 μm), by peptide linkages between amino acids of protein molecules (6.465 μm), and by OH groups in lactose molecules (9.610 μm). The wavelength of carbon hydrogen bond (3.47 μm) in the fatty acid has been used to help refine the fat results. It has been used both by itself and in conjunction with the ester linkage wavelength. Total solid may be determined by adding an experimentally determined factor to the percentage of fat, protein and lactose. Another technique, near infrared analysis or mid-infrared analysis (MIR) (33, 35), is a system makes use of wavebands in the overturn region (1400 to 2400 mm) of the mid-infrared spectrum. Absorption in this region approximates integral multipliers of the frequencies of

absorption in the min-infrared spectrum. Because of the many overtones for each fundamental absorption waveband, there is considerable overlapping of wavebands of difference molecular groups. Both techniques are assigned in AOAC official methods and very important in diary industry (36).

2.4.2 Vitamins

Several analytical methods are used for the determination and quantification of vitamins A and E contents. The current method of analysis for vitamins A and E content listed by AOAC official methods (36) is the Carr-Price colorimetric method. Senyk *et al*. (37) and Thompson et al. (38) developed a fluorometric method for milk products. They claimed that their methods eliminated interference by saponification, required a small sample, and yield reproducible and accurate results. There are normal-phase high performance liquid chromatography (NP-HPLC) (20), reversed-phase high-performance liquid chromatography (RP-HPLC) (5, 25, 39-43), gas chromatography (44), supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) (45-47). For vitamin B_1 and folic acid, current AOAC official methods (36) for the determination of thiamine (B_1) and folic acid content using chemical and microbiological techniques. Nowadays, the analytical methods used for determination of vitamin B_1 and folic acid in food are reversed-phase high-performance liquid chromatography (RP-HPLC) is widely use (48-52).

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

Name	Grade	Source/Supplier
Acetic acid, glacial	AR	Lab-Scan, Thailand
Ascorbic acid	AR	Merk, Germany
Butylated hydroxytoluene	AR	Sigma, Germany
Ethanol, absolute	AR	Merk, Germany
Ether, diethyl	AR	Labscan, Thailand
Ether, petroleum	AR	Labscan, Thailand
Folic acid	AR	Fluka, Switzerland
Meta-phosphoric acid	AR	Fluka, Switzerland
Methanol	HPLC	Labscan, Thailand
Sodium 1-heptane-sulfonate	HPLC	Sigma Aldrich,Germany
Sterile water		Thai Otsuka Phamaceutical, Thailand
α-tocopherol	AR	Fluka, Switzerland
Trichloroacetic acid	AR	Riedel-delHaën, Germany
Triethylamine	AR	Carlo Erba, Italy
Vitamin-A-palmitate	AR	Fluka, Switzerland
Vitamin B_1 hydrochloride	AR	Fluka, Switzerland

3.1.2 Instruments

Name	Source/Supplier
Analytical balance AL204	Mettler Toledo, USA
Column ODS-2 HYPERSIL 5µ 4.6 mm x 25 cm	Thermo, USA
Filter apparatus	Duran, Germany
High Performance Liquid Chromatography - Communication Bus Module CBM-10A - Liquid Chromatograph LC-10AD - UV-VIS Detector SPD-10A - Data Processing (class-LC10)	Shimadzu corporation, Japan
MilkoScan 133B	N.Foss Electric, Denmark
Nylon syringe filter (0.45-µm), 13 mm	Vertical Chromatography, Thailand
Nylon filter (0.45-µm), 46 mm	Vertical Chromatography, Thailand
Pipetman 5-ml, 1-ml, 200µl 100-µL, 200-µL and 1000-mL	Gilson Medical Electronics, France
Single tablet machine with 16 mm diameter punches and die set	Fette Eacta, Germany
Sonicator	Branson, USA
Spray Dryer	NIRO, Denmark

3.1.3 Milk samples

Pasteurized goat milk samples were purchased from supermarkets in Bangkok.

3.2 Methods

3.2.1 Production of goat milk powder

Pasteurized goat milk was concentrated to have 30% of total solid in a vacuum evaporator. After that it was taken to spray drying at 180±2 ˚C of inlet air temperature and 90±5 ˚C of outlet air temperature with feeding rate at 7-10 rpm. Goat milk powder was collected in a glass bottle then it was taken to produce goat milk tablets.

3.2.2 Production of goat milk tablets

The main ingredients of goat milk tablets were 60% spray dried goat milk powder, 16% lactose, 17% sucrose and 0.86% magnesium stearate (MgSt). Spray dried goat milk powder, lactose and magnesium stearate were passed through a 120-mesh screen. Sucrose was prepared at a concentration of 65% w/v to use as binder solution. Spray dried goat milk powder and lactose were manually mixed by gradually added binder solution. The damp mass was passed through a 14-mesh screen for making granules. The granules were dried in hot air oven (50 ^{0}C) for 3 hours. After that 0.86% magnesium stearate was added into the granules and it was continuously mixed for 5 min. The tablets were compressed on a single punch tablet machine using 16-mm punch. The machine was adjusted to have a tablet weight of about 1 g.

3.2.2.1 Evaluation of tablet properties

a) Weight variation

Weight variation test was determined by weighing 20 tablets, individually, calculating the average weight and comparing the individual tablet weight to the average.

b) Thickness and hardness

Twenty goat milk tablets were sampling and determined for thickness (mm), and hardness (kg) using an electronic thickness/hardness tester (Pharma test® Model DTB 311, Pharmatest, Germany)

c) Friability

Twenty goat milk tablets were brushed to remove dust and then weighed (W_1). The tablet samples were placed in a Roches friabilator (Pharma test® Model PTFR-A, Pharmatest, Germany) as set rotating at 25 rpm for 4 min. After that the tablet samples were brought out and weighed again (W_2). The friability value (F %) of tablet was calculated from the different weigh of goat milk tablet before and after rotating, which was calculated from the following equation:

$$F\ \% = [(W_1 - W_2)/ W_1] \times 100$$

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3.2.3 Storage of milk samples

Goat milk tablets were separately kept in high density polyethylene plastic bottles and aluminum laminated polyethylene pouches stored at room temperature and accelerated conditions (45°C and 90% relative humidity). The samples were taken for analysis after 0, 15, 30, 45, 60, 75, and 90 days of storage.

3.2.4 Analysis of composition of milk samples

The goat milk tablets were grinded to powder. An accurate weight of the powder, about 3.0 g, and 30 mL of distilled water was added and mixed until complete homogenization. Then it was preheated to 40 °C to melt the fat before analysis. Air was incorporated to the samples by vigorous mixing just before analysis by MilkoScan. Each milk sample was performed in duplicate.

3.2.5 Analysis of vitamins A and E in milk sample

3.2.5.1 Instrumentation

Determination of vitamins A and E were carried out using a High Performance Liquid Chromatography system (Shimadzu corporation, Kyoto, Japan) equipped with Liquid Chromatograph LC-10 AD, Communication Bus Model CBM-10A, UV-Visible Detector SPD-10A and Data processing (class LC-10). The separation was performed on a ODS-2 HYPERSIL C18 Column 250 x4.6 mm, 5 µm. (Thermo, USA). Manual injection was carried out using a Rheodyne model 7725 injector with a 20- µL loop.

The mobile phase for the chromatographic separation consisted of methanol and *n*-hexane. The mobile phase was filtered through a nylon membrane (47 mm, 0.45 μm) and degassed by sonication. Isocratic flow rate of 1 mL/min was maintained. Vitamins A and E were detected by UV-VIS spectrophotometer at 295 nm.

3.2.5.2 Preparation of standard solution

Stock standard solutions of vitamin-A-palmitate and α-tocopherol containing 100 mg/l was prepared in 100% ethanol and stored at −20 °C in dark bottles for not more than one month. Working standard solutions were prepared from these solutions and diluted with ethanol prior to analysis.

3.2.5.3 Sample preparation

Goat milk tablets were finely powdered. Four grams of powder sample was reconstituted with 8 ml of double-distilled water. It was then immersed in warm water (40 °C), and mixed until complete homogenization. Two milliliters of reconstituted sample was transferred to a centrifuge tube. Five milliliters of 0.1% (w/v) ascorbic acid and 2 mL of 50% (w/v) potassium hydroxide solution were added. The mixture was again stirred for 1 min and placed in a water bath (80 ^{0}C) for 20 min. After that it was cooled and placed in an ice water bath. Twenty milliliters of 0.01% (w/v) of butylated hydroxytoluene in diethyl ether and petroleum ether (1:1) were added. The mixture was vortexed for 1 min and centrifuged at 3500*g* for 15 min to aid solvent separation. The 10 ml of clear organic top layer was removed and evaporated to dryness under nitrogen. The extract was reconstituted with 1 mL of methanol and then filtered with a 0.45 μm nylon filter before injection into HPLC.

3.2.5.4 Optimization

Preliminary experiment for the separation of vitamins A and E was modified from Joanna Karpińska *et al.* method (38). The optimum HPLC conditions for the separation of vitamins A and E were determined from several analytical parameters including resolution (> 1.5), tailing factor (close to 1.0) and number of theoretical plates (> 5,000).

The ratio of methanol and *n*-hexane was investigated. Mixtures of methanol and *n*-hexane at the ratio 80:20, 85:15, 90:10 and 95:5 v/v were prepared as mobile phases and filtered through a 0.45-μm membrane filters.

3.2.5.5 Validation of HPLC method

The developed method was validated for the quantitative analysis of vitamins A and E in goat milk tablets. The parameters to be considered in the method validation study are linearity (r^2), accuracy (recoveries), precision (%RSD), limit of detection (LOD) and limit of quantitation (LOQ).

(1) Linearity

Five final concentrations of standard solutions in the range of 0.025 to 1.0 µg/mL of vitamin E and 0.1 to 2 µg/mL of vitamin A were used for linearity test. The standard calibration plot was constructed by least-square linear regression of the peak areas of vitamin A or E versus concentration.

(2) Precision

Precision was determined by the percent relative standard deviation (%RSD) of intra-day, inter-day and injection precision. For intra-day precision, three different concentrations of working standard, 0.1, 0.5 and 2 µg/mL of vitamin E and 0.025, 0.1 and 1.0 µg/mL of vitamin A were analyzed. Each concentration was injected in triplicates on the same day. For inter-day precision, the same three different concentrations of the working standard as intra-day were analyzed on six different days. Each concentration was injected in triplicates. Injection precision was performed by ten injections of the middle point concentration of calibration curve, 0.5 µg/mL of vitamin E and 0.1 µg/mL of vitamin A.

The percent relative standard deviations (%RSD), was determined from the following equation:

$$\%RSD = \frac{SD \times 100}{\overline{X}}$$

where,

SD = the standard deviation from the mean value;

$\overline{X}$ = the mean value.

The value (%RSD) should be less than 2.0 % (53).

(3) Accuracy

The accuracy was determined by the standard addition method. The standard of vitamin E in concentration 0.1, 0.5, 2 µg/mL and the standard of vitamin A in concentration 0.025, 0.1, 1 µg/mL were added into samples and analyzed. Each concentration was done in triplicate. Accuracy of method was calculated from percent recoveries by the following equation.

$$\% \text{ Recovery} = \left(\frac{X_t - X_s}{S} \right)$$

where: X_t = total vitamin concentration in spiked milk sample;

X_s = vitamin concentration in milk sample;

S = standard vitamin concentration ;

The value (%Recovery) should be within 80-110% at each level (53).

(4) Limit of detection (LOD)

LOD was calculated by the following equation (53)**;**

$$LOD = 3.3 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

(5) Limit of quatitation (LOQ)

LOQ was calculated by the following equation (53)**;**

$$LOQ = 10 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

3.2.6 Analysis of Vitamin B_1 and folic acid in milk sample

3.2.6.1 Instrumentation

A High Performance Liquid Chromatography system (Shimadzu corporation, Kyoto, Japan) equipped with Liquid Chromatograph LC-10 AD, Communication Bus Model CBM-10A, UV-Visible Detector SPD-10A and Data processing (class LC-10) was used. The analytical column was ODS-2 HYPERSIL C18 Column 250x4.6mm, 5µm. (Waters, USA). Manual injection was carried out using a Rheodyne model 7725 injector with a 20 µL loop.

The mobile phase consisted of 5 mM of sodium 1-heptane-sulfonate, 0.5% triethylamine, 2.5% glacial acetic acid and methanol. The pH was adjusted to 3.8 by acetic acid. The mobile phase was filtered through a nylon membrane (47 mm, 0.45 µm) and degassed by sonication. The analysis was carried out using an isocratic pump at a flow rate of 1.0 ml/min. The UV-VIS detector was operated at a wavelength of 265 nm.

3.2.6.2 Preparation of standard solutions

A 100 µg/mL stock standard solutions of Vitamin B_1 in 2.4% (v/v) aqueous acetic acid and 100 µg/mL stock standard solutions of folic acid in 0.1M sodium hydroxide were prepared. Working standard solutions were prepared by diluting the stock standard solution with distilled water.

3.2.6.3 Sample preparation

Goat milk tablets were finely powdered. Four grams of powder sample was reconstituted with 8 ml of double-distilled water. The mixture was then immersed in warm water (40 °C), and mixed until complete homogenization. Four milliliters of reconstituted sample was transferred to a centrifuge tube. Then 3 ml of 1% (w/v) meta-phosphoric acid was added. The mixture was thoroughly vortexed for 1 min and centrifuged at 3,500g for 10 min to separate the two phases. After that 2 ml 1% (w/v) meta-phosphoric was added to the solid residue obtained, mixed thoroughly for 1 min and centrifuged. The two acid extracts were combined in a 10-ml volumetric flask and adjusted to volume with double-distilled water. The samples were filtered through 0.45-µm membranes prior to HPLC analysis.

3.2.6.4 Optimization

Preliminary experiment for the separation of folic acid and vitamin B_1 was modified from Albalá-Hurtado *et al.* (46). The effects of organic phase ratios and pH of buffer solution on HPLC separation were investigated. The optimum HPLC condition for the separation of folic acid and vitamin B_1 were determined from several analytical parameters including resolution (> 1.5), tailing factor (close to 1.0) and number of theoretical plate (> 5,000).

The initial condition was optimized by varying the organic phase ratio (20, 15 and 10%) and pH of buffer solution (3.6, 3.7 and 3.8). The mobile phases was prepared and filtered through a 0.45-μm membrane filter.

3.2.6.5 Validation of HPLC method

(1) Linearity

Five different concentration of standard solution before injection in the range of 0.025 to 1 μg/mL of folic acid and 0.05 to 2 μg/mL of vitamin B_1 were used for linearity test. The standard calibration plot was constructed by least-square linear regression of the peak areas of vitamin B_1 or folic acid versus concentration.

(2) Precision

Precision was determined by the percent relative standard deviation (%RSD) of intra-day, inter-day and injection precision. For intra-day precision, three different concentrations of working standard, 0.05, 0.25, 1 μg/mL of folic acid and 0.1, 0.5, 2 μg/mL of vitamin B_1 were analyzed and each concentration was injected in triplicates on the same day. For inter-day precision, the same three different concentrations of the working standard as intra-day were analyzed on six different days and each concentration was injected in triplicates. Injection precision was performed by ten injections of the middle point concentration of calibration curve, 0.25 μg/mL of folic acid and 0.5 μg/mL of vitamin B_1.

The percent relative standard deviations (%RSD), which was determined from the following equation:

$$\%RSD = \frac{SD \times 100}{\overline{X}}$$

where,

SD = the standard deviation from the mean value;

$\overline{X}$ = the mean value.

The value (%RSD) should be less than 2.0 % (53).

(3) Accuracy

The accuracy was determined using the standard addition method. The standard folic acid in concentration 0.05, 0.25, 1µg/mL and vitamin B_1 in concentration 0.1, 0.5, 2 µg/mL were added into samples and analyzed. Each concentration was done in triplicate. Accuracy of method was calculated from percent recoveries by the following equation.

$$\% \text{ Recovery} = \left(\frac{X_t - X_s}{S} \right)$$

where: Xt = total vitamin concentration in spiked milk sample;

Xs = vitamin concentration in milk sample;

S = standard vitamin concentration.

The value (%Recovery) should be within 80-110% at each level (53).

(4) Limit of detection (LOD)

LOD was calculated by the following equation (53);

$$LOD = 3.3 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

(5) Limit of detection (LOD)

LOD was calculated by the following equation (53);

$$LOQ = 10 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

3.2.7 Statistical analysis

The data for the component in milk samples were analyzed using t-test with the 95% confidence interval ($p < 0.05$). The factors included in the models were material (high density polyethylene plastic bottle and aluminium laminated pouch) and storage time (0, 15, 30, 45, 60 and 90 days).

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Production of goat milk tablet

The physical appearances of goat milk tablets which obtained by wet granulation method were canary yellow with shiny and smooth surface. The photograph of goat milk tablet is shown in Figure 1. The average weight of goat milk tablets was 1.0394±0.01 g and the weight variation met the requirement of USP 24 (54). The thickness and hardness of the tablets were 4.497±0.07 mm and 5.75±0.67 kg, respectively. Additionally, the friability of tablet sample was 0.36 % which met the requirement of USP 24 which stated that the friability of tablet should not more than 1% (54).

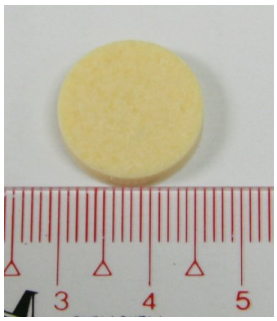


Figure 1 Photograph of goat milk tablet

4.2 Analysis of composition of milk samples

The compositions of goat milk tablets were analyzed by MilkoScan. The contents of fat, protein, lactose and solid-not-fat which stored at room temperature and accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches are shown in Tables 5 and 6. At both storage condition, room temperature storage and accelerated storage, the contents of fat, protein, lactose

Table 5 Compositions of goat milk tablets during storage in two different packaging at room temperature (n=2)

Storage of time (days)	Plastic bottle				Aluminium laminated pouch			
	Content (g/100g)				Content (g/100g)			
	Fat	Protein	Lactose	Solid-not-fat	Fat	Protein	Lactose	Solid-not-fat
0	60.21±0.34	47.06±0.42	145.94±2.07	214.66±0.73	60.21±0.34	47.06±0.42	145.94±2.07	214.66±0.73
30	59.83±0.47	47.58±0.36	145.67±1.18	213.75±4.36	60.50±0.24	47.17±0.23	149.75±0.60	214.34±3.06
60	59.16±0.66	47.84±0.23	146.17±1.65	214.33±0.71	60.92±0.12	47.75±0.11	148.58±0.12	214.83±2.36
90	59.67±0.23	47.75±0.11	146.58±0.35	214.00±0.47	59.16±0.23	47.83±00	148.91±0.35	214.42±0.35

Table 6 Compositions of goat milk tablets during storage in two different packaging at accelerated conditions (45°C and 90% relative humidity) (n=2)

Storage of time (days)	Plastic bottle				Aluminium laminated pouch			
	Content (g/100g)				Content (g/100g)			
	Fat	Protein	Lactose	Solid-not-fat	Fat	Protein	Lactose	Solid-not-fat
0	60.21±0.34	47.06±0.42	145.94±2.07	214.66±0.73	60.21±0.34	47.06±0.41	145.94±2.07	214.66±0.73
30	60.42±0.36	48.50±0.95	145.75±0.60	214.25±1.77	60.25±0.35	48.42±0.35	148.67±2.12	214.92±2.24
60	60.66±0.47	48.17±0.71	143.92±0.59	215.83±0.23	60.25±0.59	49.17±0.47	147.33±0.24	216.42±2.10
90	59.50±0.40	47.67±0.71	145.58±0.35	215.49±2.36	59.83±0.24	48.42±0.35	147.59±0.87	214.00±0.94

and solid-not-fat were unchanged after storage in both containers for 90 days. From statistical analysis, the effect of plastic bottles and aluminium laminated pouches containers on composition of milk were not significantly different ($P > 0.05$).

There are little information exist in literatures about fat content change during storage in milk powder. Free fatty acid could be produced by hydrolysis of fat in milk. The bacterial thermostable lipases are important in free fatty acid production during the production and storage of milk powder (55). However, infrared analysis by MilkoScan indicated no significant decrease in fat content during storage for 90 days because free fatty acid could also be detected by MilkoScan.

Protein-protein or protein-carbohydrate interactions can cause the loss of nutritive value. The result from that was lowering of the availabilities of the sulphur amino acid and lysine (56). In addition, the protein change in milk might be caused by native or bacterial proteinases. Proteolytic enzymes were able to hydrolyze protein to free amino acid during storage (57). However, the study by Thiangthum and Ruangwises (32) showed no significant changes for protein in UHT milk during storage, which supported no change of protein content as we obtained in our study.

The change of lactose in milk could be occurred by the formation of tautomerization. The tautomerized product is lactulose (58). MilkoScan can measure the lactose content that the summation of lactose and lactulose contents. Accordingly, the lactose content in our study did not change during storage.

Theoretically, solid-not-fat content was the summation of protein, lactose, vitamins and other nutrients content, except fat content. So, solid-not-fat content was calculated from fat, protein and lactose contents which were measured by MilkoScan. Since, fat, protein and lactose contents in milk sample did not change during storage. The solid-not-fat content in this study did not change.

4.3 Analysis of vitamins A and E contents in milk samples

The contents of vitamins A and E were determined following the chromatography method described by Joanna Karpińska *et al.* (42) with modification of the HPLC mobile phase to a mixture of methanol- *n*-hexane 90:10 (v/v). The optimized condition provided the baseline separation for vitamins A and E within 12 min with the resolution of 18.15. The number of theoretical plate was more than 9101 and the tailing factors were 1.15 and 1.06, for vitamins A and E, respectively.

4.3.1 Method validation

(1) Linearity

The calibration curves of vitamin A and vitamin E were established by triplicate injections of five different concentrations of the working standard solutions. They were evaluated by the correlation coefficients. From Table 7, the calibration curves of both vitamins A and E provided acceptable correlation coefficients ($r^2 > 0.999$). The HPLC chromatogram of standard vitamins A and E is shown in Figure 2.

Table 7 Slope, y-intercept, standard error of slope and intercept and correlation coefficients of vitamins A and E

Analyte	Range (µg/mL)	Slope	y-intercept	Standard error of mean		r^2
				Slope	Intercept	
Vitamin A	0.025-1	45630	+51.31	217.1842	129.5859	0.9999
Vitamin E	0.1-2	14601	-331.09	190.8552	126.5226	0.9998

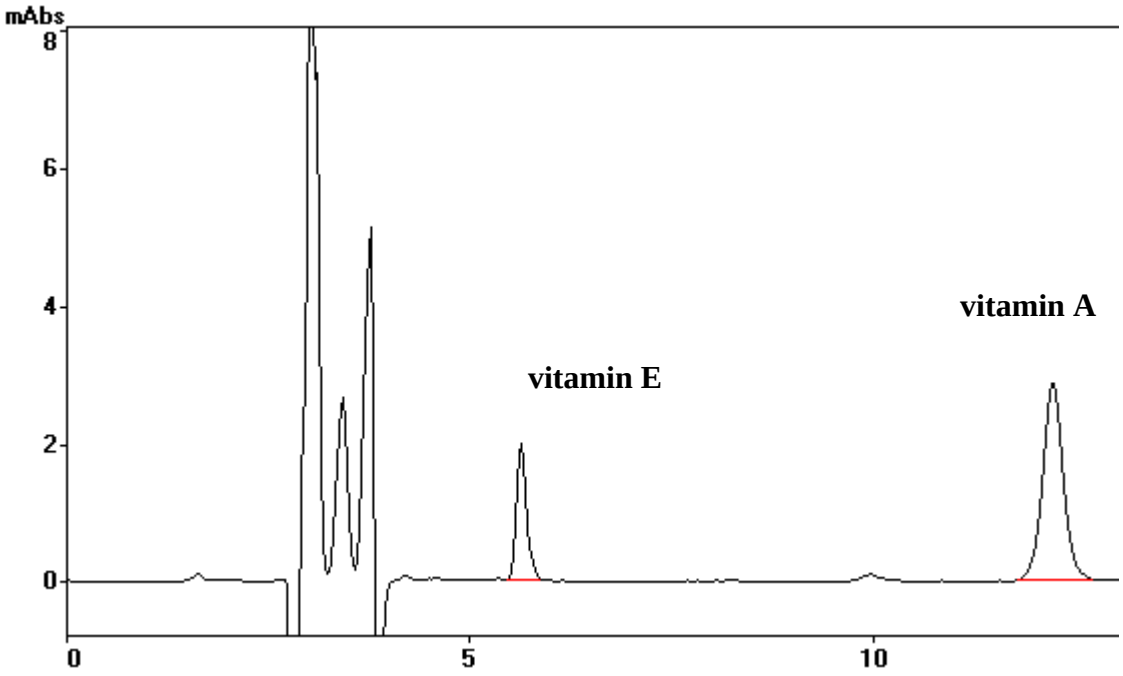


Figure 2 The HPLC chromatogram showing retention time of standard vitamin A, vitamin E. Condition: the analytical column was an ODS-2 HYPERSIL column (5 μm, 4.6-mm I.D. x 250 mm). The mobile phase consisted of *n*-hexane (eluent A) and methanol (eluent B) (10:90, v/v). The isocratic flow rate was 1 mL/min. The UV-VIS detection wavelength was 295 nm. The injection volume was 20 μL.

(2) Precision

The intra-day, inter-day and injection precision of the analytical procedure were performed. The data was expressed as percentage of relative standard deviation (%RSD) of retention time and peak area which shown in Table 8. The %RSD of injection precision of retention time (t_R) and peak area (Area) for both vitamins A and E were not more than 0.08 and 1.50, respectively. The intra-day precision, %RSD of retention time and peak area were not more than 0.75 and 1.79, respectively. For inter-day precision, the %RSD of retention time and peak area were not more than 0.14 and 1.35, respectively. All of the precision results were complied with AOAC (53) requirement (%RSD less than 2.0).

Table 8 Precision of vitamins A and E presented as %RSDs

Analyte	**Concentration**	**Intra-day**		**Inter-day**		**Injection**	
	µg/mL	t_R	**Area**	t_R	**Area**	t_R	**Area**
Vitamin A	0.025	0.15	0.94	0.13	0.73	-	-
	0.1	0.21	1.79	0.09	0.96	0.08	0.54
	1	0.10	0.12	0.08	0.90	-	-
Vitamin E	0.1	0.20	1.78	0.14	1.35	-	-
	0.5	0.23	0.45	0.09	0.79	0.04	1.50
	2	0.75	0.14	0.12	0.27	-	-

(3) Accuracy

The accuracy of the method was determined by standard addition method. The results expressed as percent recoveries are shown in Table 9. Percent recoveries of vitamins A and E were found in ranges of 84.40-92.15% and 88.67-95.16%, respectively. All of the recoveries results were in compliance with the AOAC (53) requirement (80-110%).

Table 9 Recovery data of vitamins A and E (n=3)

Analyte	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery
Vitamin A	0.025	0.0207	84.40
	0.1	0.0899	89.87
	1	0.9215	92.15
Vitamin E	0.1	0.0887	88.67
	0.5	0.4670	93.41
	2	1.9032	95.16

(4) Limit of detection (LOD) and limit of quantitation (LOQ)

The parameters LOD and LOQ of vitamins A and E were determined on the basis of response and slope of the regression equation. The following equation was used to calculate LOD.

$$LOD = 3.3 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

The calculated LOD values for vitamins A and E were 0.0094 and 0.0286 μg/mL, respectively.

For LOQ, the following equation was used to calculate.

$$LOQ = 10 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

The calculated LOQ values for vitamins A and E were 0.0285 and 0.0866 μg/mL, respectively.

4.3.2 Determination of vitamins A and E in goat milk tablets

The quantitative analysis vitamins A and E were determined using external standard method. Before application to HPLC system the samples were prepared as described in sample preparation. Tables 10 and 11 show the vitamin A and E contents of goat milk tablets stored at room temperature and accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches. The chromatogram of vitamins A and E in goat milk tablet samples is shown in Figure 3.

Table 10 Vitamins A and E contents of goat milk tablets during storage in plastic bottles and aluminium laminated pouches at room temperature (n=3)

Storage time(days)	Content of vitamin A (µg/g)		Content of vitamin E (µg/g)	
	Plastic bottle	Aluminium laminated pouch	Plastic bottle	Aluminium laminated pouch
0	0.0780	0.0780	0.1000	0.1000
15	0.0777	0.0778	0.1000	0.1003
30	0.0773	0.0777	0.1001	0.1000
45	0.0771	0.0777	0.1000	0.1000
60	0.0766	0.0772	0.1000	0.1001
75	0.0769	0.0771	0.0999	0.1001
90	0.0767	0.0771	0.1000	0.1002

The initial contents of vitamins A and E were 0.0780 and 0.1000 µg/g, respectively. The contents of vitamins A and E in goat milk tablets during room temperature storage are shown in Figures 4 and 5, respectively. Vitamin E content was unchanged after storage for 90 days in both type of containers. The content of vitamin A throughout the storage time at room temperature was decreased for 1.73% and 1.19% in plastic bottles and aluminium laminated pouches, respectively. It was not statistically significant ($p > 0.05$). Thus, vitamin A and vitamin E contents could be considered constant throughout the 90 days of storage at room temperature in both containers. The results agreed with those reported in a previous study of the stability of powder infant milk which stored at 20 and 30 ^{0}C (5). The content of vitamin A in goat milk tablets which kept in plastic bottles was decreased more than in aluminium laminated pouches but it was not significantly different ($P > 0.05$).

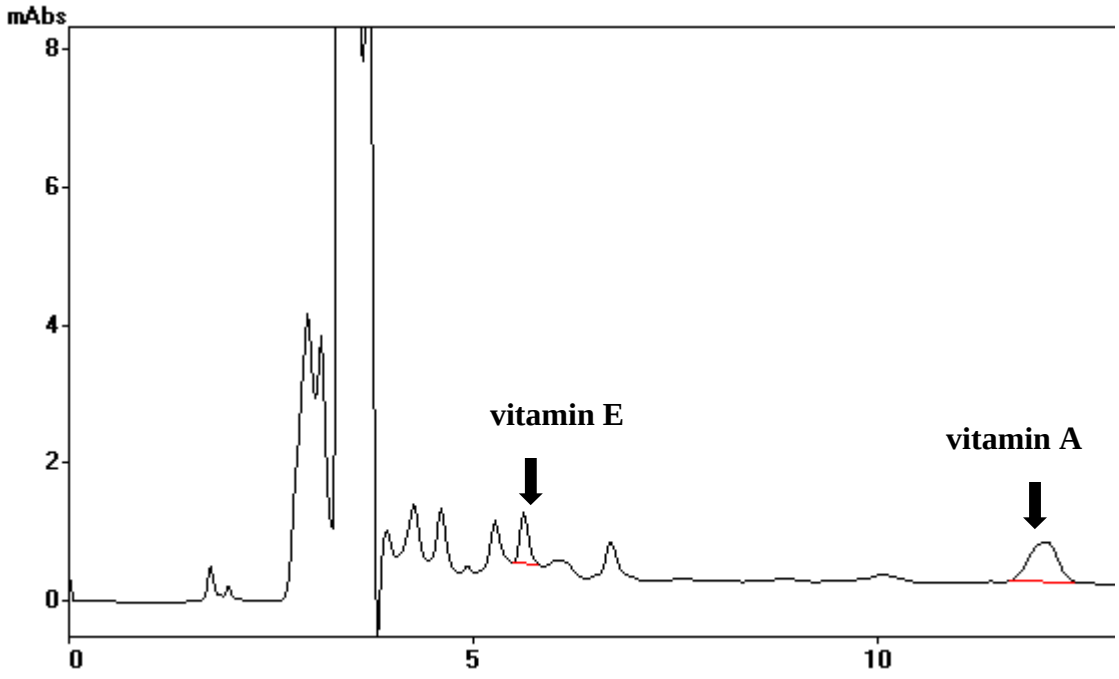
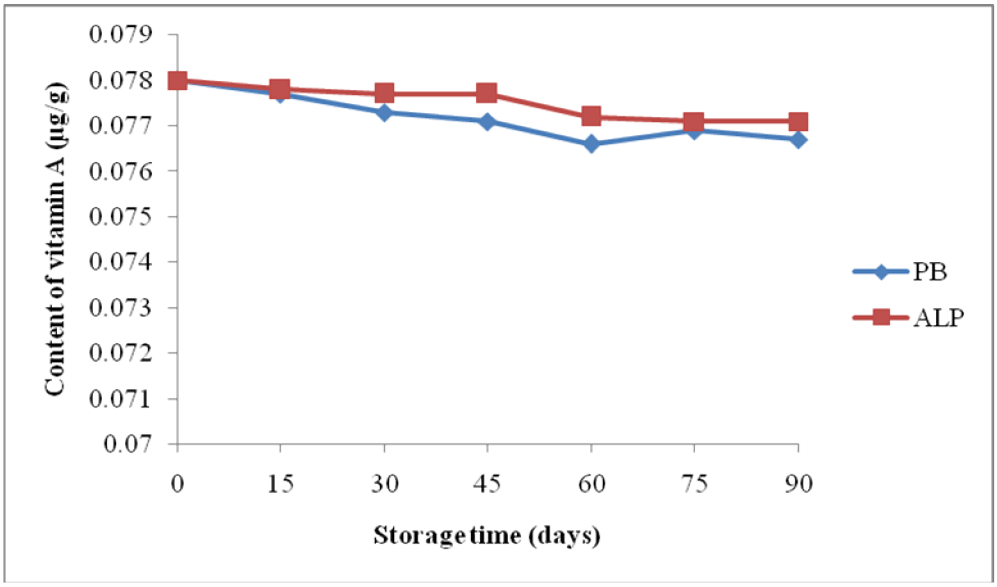
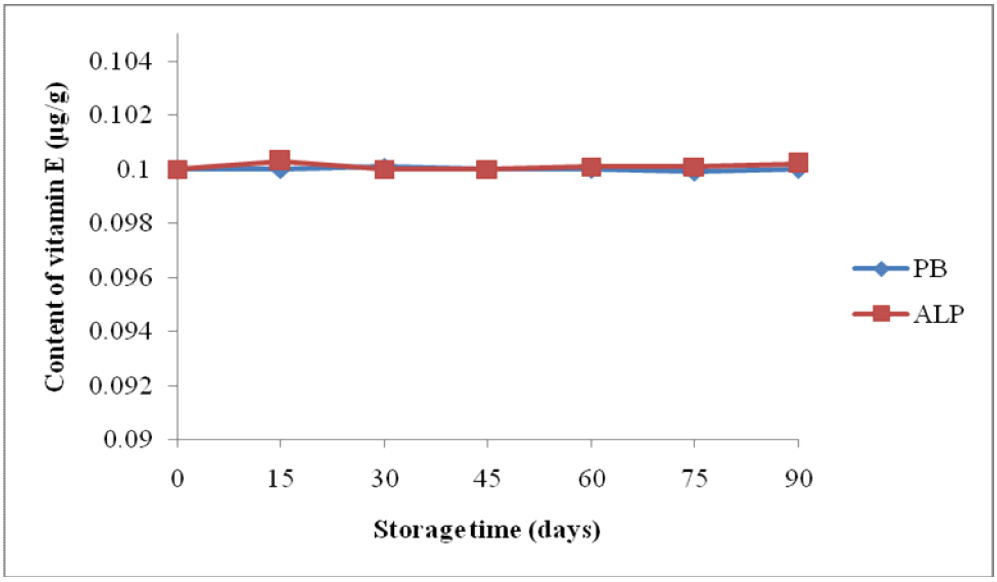


Figure 3 The HPLC chromatogram showing retention time of standard vitamin A, vitamin E in goat milk tablet samples. Condition: the analytical column was an ODS-2 HYPERSIL column (5 μm, 4.6-mm I.D. x 250 mm). The mobile phase consisted of *n*-hexane (eluent A) and methanol (eluent B) (10:90, v/v). The isocratic flow rate was 1 mL/min. The UV-VIS detection wavelength was 295 nm. The injection volume was 20 μL.



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 4 Vitamin A content of goat milk tablets during storage at room temperature in plastic bottles and aluminium laminated pouches



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 5 Vitamin E content of goat milk tablets during storage at room temperature in plastic bottles and aluminium laminated pouches

Table 11 Vitamins A and E contents of goat milk tablets during storage in plastic bottles and aluminium laminated pouches at accelerated condition (45°C and 90% relative humidity) (n=3)

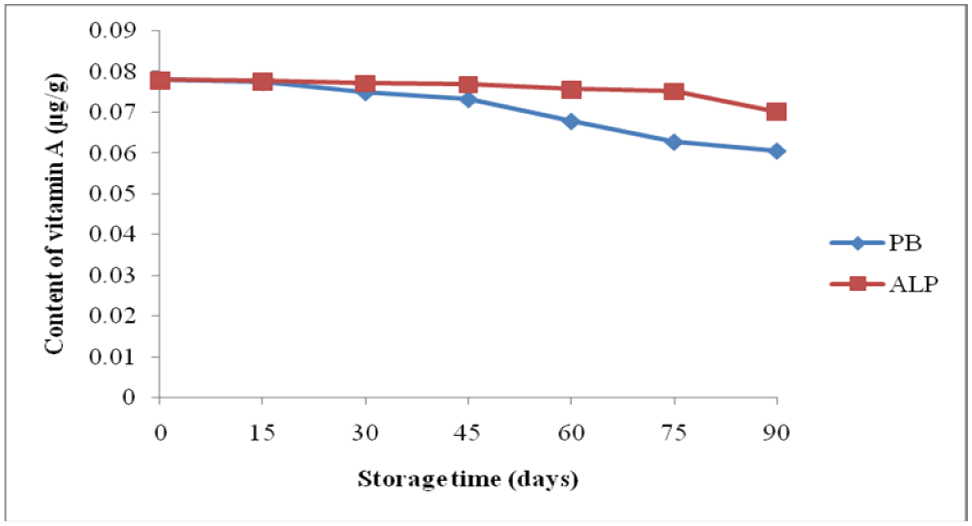
Storage time(days)	Content of vitamin A (µg/g)		Content of vitamin E (µg/g)	
	Plastic bottle	Aluminium laminated pouch	Plastic bottle	Aluminium laminated pouch
0	0.0780	0.0780	0.1000	0.1000
15	0.0775	0.0777	0.0994	0.0994
30	0.0750	0.0772	0.0984	0.0990
45	0.0733	0.0770	0.0978	0.0986
60	0.0679	0.0757	0.0956	0.0981
75	0.0628	0.0753	0.0915	0.0974
90	0.0606	0.0702	0.0879	0.0958

The contents of vitamin A and E in goat milk tablets which stored at accelerated condition were shown in Table 11. Change of contents of vitamins A and E in goat milk tablets during accelerated storage are shown in Figures 6 and 7, respectively. After 90 days, losses of vitamin A in plastic bottles and aluminium laminated pouches were 22.34 and 10.00%, respectively. The contents of vitamin E throughout storage at accelerated conditions reduced for 12.15 and 4.22% in goat milk tablets kept in plastic bottles and aluminium laminated pouches, respectively. Though the decreasing of vitamin A and E contents of goat milk tablets in plastic bottles was more than that in aluminium laminated pouches. It was not significantly different ($P > 0.05$).

Kinetic modeling is necessary to derive basic information for a system in order to describe the reaction rate as a function of storage time. A reaction rate expression for the degradation kinetics can be written as the below equations;

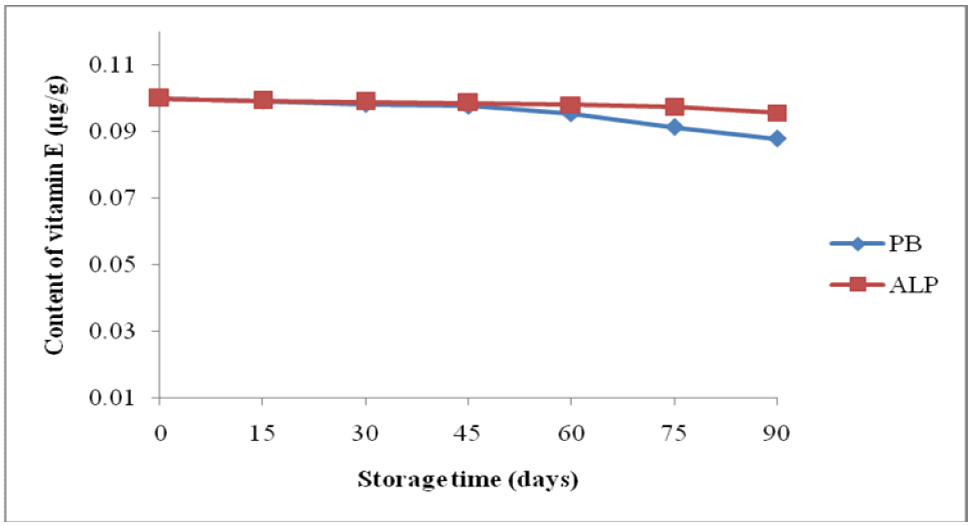
$C = C_0 \pm k_0 t$ for zero order degradation reaction kinetic

and $\ln C = \ln C_0 \pm k_1 t$ for first order degradation reaction kinetic (59).



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 6 Vitamin A content of goat milk tablets during storage at accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 7 Vitamin E content of goat milk tablets during storage at accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches

Where (+) and (-) indicate the formation and the degradation of quality parameters, respectively. In order to determine the rate of change of vitamins A and E, the kinetics of these parameters were investigated. It was investigated for each parameter follows either zero or first order kinetics. Kinetic data were analyzed by regression analysis using MS excel. Tables 12 and 13 show the kinetic parameters obtained from the assumption of zero and first order kinetics. It can be observed on the basis of correlation coefficients (R) that vitamins A and E followed zero order degradation reaction kinetic.

Table 12 Non-linear regression analysis of vitamins A and E from zero order and first order reaction kinetics of goat milk tablets in plastic bottles

Parameter	Zero order			First order		
	K_0	C_0	R	K_1	C_0	R
Vitamin A	-0.0008	0.3209	0.9459	-0.0030	-1.1301	0.9367
Vitamin E	-0.0005	0.4068	0.8811	-0.0014	-0.8977	0.8720

Table 13 Non-linear regression analysis of vitamins A and E from zero order and first order reaction kinetics of goat milk tablets in aluminium laminated pouches

Parameter	Zero order			First order		
	K_0	C_0	R	K_1	C_0	R
Vitamin A	-0.0002	0.3129	0.9340	-0.0010	-1.1502	0.7140
Vitamin E	-0.0002	0.4008	0.9305	-0.0004	-0.9141	0.9277

4.4 Analysis of vitamin B_1 and folic acid contents in milk samples

Folic acid and vitamin B_1 contents in milk samples were analyzed using reverse-phase liquid chromatography method as described by Albalá-Hurtado *et al.* (50) with modification of the ratio of methanol to 10% and 90% of buffer solution (5 mM sodium 1-heptane-sulfonate, 0.5% triethylamine, 2.5% glacial acetic acid, pH 3.8). The optimized condition provided the baseline separation for folic acid and vitamin B_1 within 15 min. The resolution was 4.39 and the number of theoretical plate was more than 6825. The tailing factors were 1.27 and 1.15 for folic acid and vitamin B_1, respectively.

4.4.1 Method validation

(1) Linearity

The linearity was tested at the concentration ranges of 0.05-1 μg/mL for folic acid and 0.1-2 μg/mL for vitamin B_1. Calibration curves were constructed and evaluated by the correlation coefficient (r^2). The correlation coefficients (r^2) of all the calibration curves were greater than 0.999 (Table 14). The HPLC chromatogram of standard folic acid and vitamin B_1 is shown in Figure 8.

Table 14 Slope, y-intercept, standard error of slope and intercept and correlation coefficients of folic acid and vitamin B_1

Analyte	Range (μg/mL)	Slope	y-intercept	Standard error of mean		r^2
				Slope	Intercept	
Folic acid	0.05-1	43962	-105.82	226.7964	150.349	0.9999
Vitamin B_1	0.1-2	32832	-633.40	432.7037	286.850	0.9998

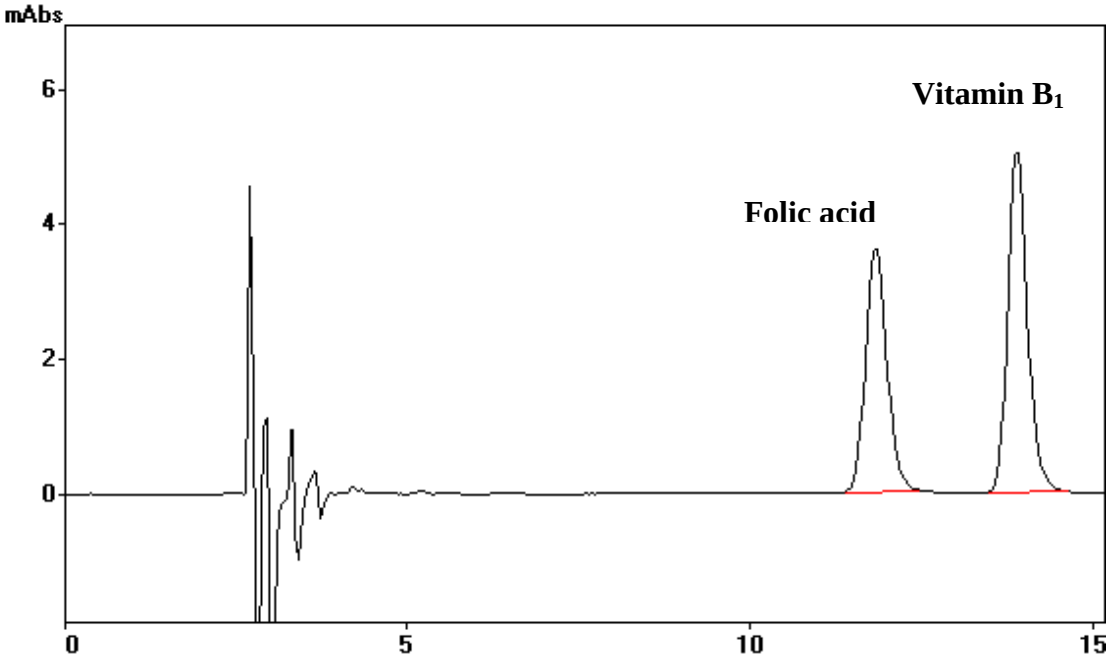


Figure 8 The HPLC chromatogram showing retention time of standard folic acid, vitamin B_1. Condition: The analytical column was an ODS-2 HYPERSIL column (5 µm, 4.6-mm I.D. x 250 mm). The mobile phase consisted of 5 mM Sodium-1-heptane-sulfonate, 0.5% triethylamine, 2.5% glacial acetic acid (eluent A) and methanol (eluent B) (90:10, v/v). The isocratic flow rate was 1 mL/min. The UV-VIS detection wavelength was 265 nm. The injection volume was 20 µL.

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(2) Precision

The intra-day, inter-day and injection precision of the analytical procedure were performed. The data was expressed as the percentage of relative standard deviation (%RSD) of retention time (t_R) and peak area (Area) (Table 15). The %RSD of injection precision of retention time and peak area for both folic acid and vitamin B_1 were not more than 0.65 and 0.59, respectively. The intra-day precision, %RSD of retention time and peak area were not more than 0.79 and 1.04, respectively for both analytes. For inter-day precision, the %RSD of retention time and peak area were not more than 0.70 and 1.33, respectively for both analytes. All of the precision results were complied with AOAC (53) requirement (%RSD less than 2.0).

Table 15 Precision of folic acid and vitamins B_1 presented as %RSDs

Analyte	Concentration	Intra-day		Inter-day		Injection	
	µg/mL	t_R	Area	t_R	Area	t_R	Area
Folic acid	0.05	0.23	1.04	0.67	1.32	-	-
	0.25	0.79	0.66	0.31	1.22	0.65	0.57
	1	0.46	0.18	0.13	1.33	-	-
Vitamin B_1	0.1	0.15	0.31	0.70	1.00	-	-
	0.5	0.12	0.68	0.46	0.38	0.14	0.59
	2	0.10	0.19	0.67	0.50	-	-

(3) Accuracy

The accuracy of the method was determined by standard addition method. The goat milk tablet samples were spiked with standard folic and standard vitamin B_1 at three concentrations represents the low, medium and high concentrations of the calibration curves. The results expressed as percent recoveries are shown in Table 16. Percent recoveries of folic acid and vitamin B_1 were in a range of 80.67-90.38 % and 89.25-96.40%, respectively. All of the recoveries results are in compliance with the AOAC (53) requirement (80-110%).

Table 16 Recovery data of folic acid and vitamin B_1 (n=3)

Analyte	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery
Folic acid	0.05	0.0405	80.67
	0.25	0.2200	88.02
	1	0.9038	90.38
Vitamin B_1	0.1	0.0894	89.25
	0.5	0.4648	92.95
	2	1.9280	96.40

(4) Limit of detection (LOD) and limit of quantitation (LOQ)

The detection limit, the lowest concentration that can be detected, were calculated using the following equation.

$$LOD = 3.3 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

The calculated LOD values for folic acid and vitamin B_1 were 0.0113 and 0.0288 μg/mL, respectively.

For LOQ, the following equation was used to calculate.

$$LOQ = 10 (SD/S)$$

Where,

SD = the standard deviation of y-intercept of calibration curve;

S = the slope of calibration curve.

The calculated LOQ values for folic acid and vitamin B_1 were 0.0342 and 0.0874 μg/mL, respectively.

4.4.2 Determination of folic acid and vitamin B_1 in goat milk tablets

The quantitative analysis folic acid and vitamin B_1 were determined using external standard method. Samples were prepared as described in sample preparation before application to HPLC system to determined folic acid and vitamin B_1. Tables 17 and 18 show folic acid and vitamin B_1 content of goat milk tablets stored at room temperature and accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches, respectively. Figure 9 shows HPLC chromatogram of folic acid and vitamin B_1 in goat milk tablet sample.

Table 17 Folic acid and vitamin B_1 contents of goat milk tablets during storage in plastic bottles and aluminium laminated pouches at room temperature (n=3)

Storage time(days)	Content of folic acid (µg/g)		Content of vitamin B_1 (µg/g)	
	Plastic bottle	Aluminium laminated pouch	Plastic bottle	Aluminium laminated pouch
0	0.0499	0.0499	0.0779	0.0779
15	0.0500	0.0497	0.0780	0.0779
30	0.0498	0.0498	0.0778	0.0780
45	0.0496	0.0496	0.0775	0.0773
60	0.0497	0.0497	0.0770	0.0772
75	0.0498	0.0497	0.0770	0.0773
90	0.0492	0.0494	0.0769	0.0770

The initial folic acid and vitamin B_1 contents of goat milk tablets were 0.0499 and 0.0779 µg/g, respectively. No change was found in the contents of folic acid and vitamin B_1 in goat milk tablets during room temperature storage in both containers. The contents of folic acid and vitamin B_1 is shown in Figures 10 and 11, respectively. The final contents of folic acid were 0.0492 and 0.0494 µg/g after 90 days of storage for goat milk tablets kept in plastic bottles and aluminium laminated pouches, respectively. After 90 days of storage, the vitamin B_1 contents of goat milk tablets in plastic bottles and aluminium laminated pouches decreased to 0.0769 and 0.0770 µg/g, respectively. The decreasing of folic acid and vitamin B_1 of goat milk tablets throughout storage at room temperature were not statistically significant ($p > 0.05$)

in both containers. The results agree with those reported in a previous study of the stability of powder infant milk which storage at 20 and 30 ^{0}C (5).

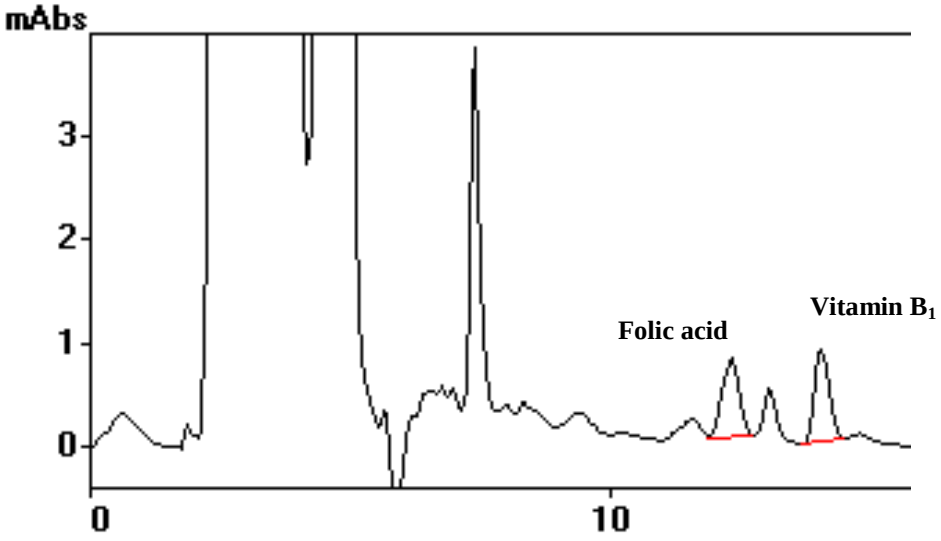
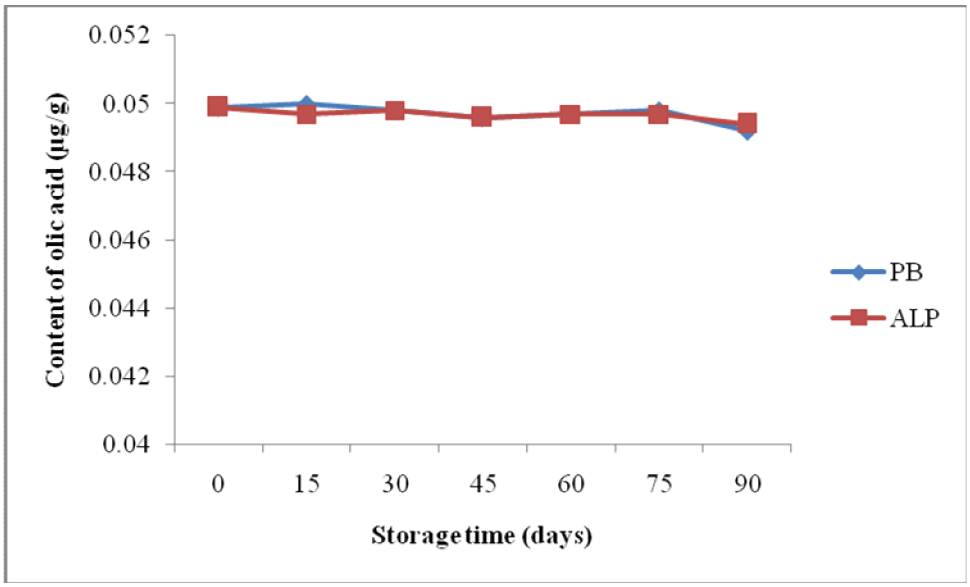
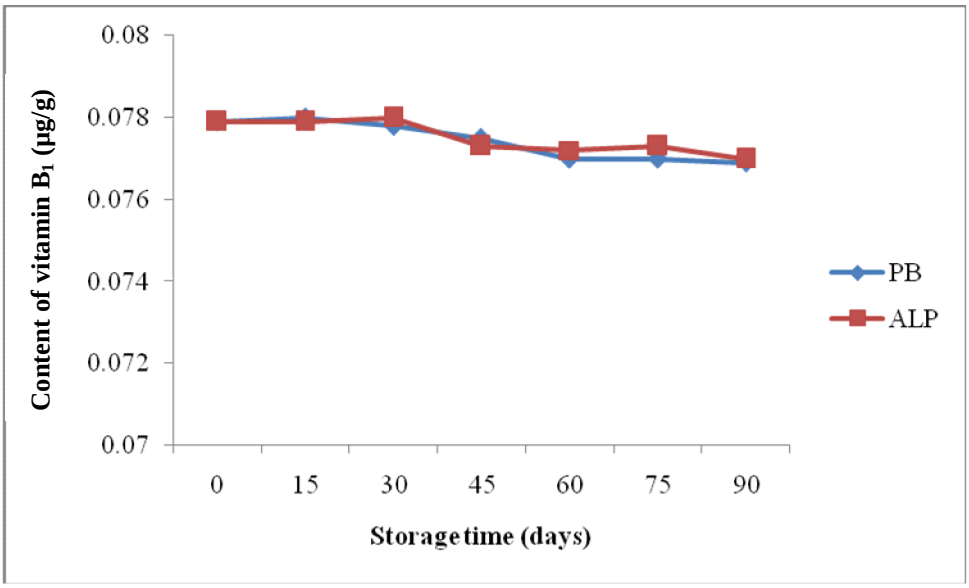


Figure 9 The HPLC chromatogram showing retention time of folic acid and vitamin B_1 in goat milk tablets sample. Condition: The analyticall column was an ODS-2 HYPERSIL (5 µm, 4.6-mm I.D. x 250 mm). The mobile phase consisted of 5 mM Sodium-1-heptane-sulfonate, 0.5% triethylamine, 2.5% glacial acetic acid (eluent A) and methanol (eluent B) (90:10, v/v). The isocratic flow rate was 1 mL/min. The UV-VIS detection wavelength was 265 nm. The injection volume was 20 µL.



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 10 Folic acid content of goat milk tablets during storage at room temperature in plastic bottles and aluminium laminated pouches



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 11 Vitamin B_1 content of goat milk tablets during storage at room temperature in plastic bottles and aluminium laminated pouches

In Table 18 the initial folic acid and vitamin B_1 contents of goat milk tablets were 0.0499 and 0.0779 µg/g, respectively. The change of contents of folic acid and vitamin B_1 in goat milk tablets during accelerated storage were shown in Figures 12 and 13, respectively. The folic acid content decreased for 74.89 and 57.98% after 90 days of storage for goat milk tablets kept in plastic bottles and aluminium laminated pouches, respectively. The vitamin B_1 content decreased to 57.54 and 41.33% after 90 days of storage for goat milk tablets kept in plastic bottles and aluminium laminated pouches, respectively. The decreasing of folic acid and vitamin B_1 was more in plastic bottles than in aluminium laminated pouches but from statistical analysis it was not significantly different ($P > 0.05$).

Table 18 Folic acid and vitamin B_1 contents of goat milk tablets during storage in plastic bottles and aluminium laminated pouches at accelerated conditions (45°C and 90% relative humidity) (n=3)

Storage time(days)	Content of folic acid (µg/g)		Content of vitamin B_1 (µg/g)	
	Plastic bottle	Aluminium laminated pouch	Plastic bottle	Aluminium laminated pouch
0	0.0499	0.0499	0.0779	0.0779
15	0.0470	0.0480	0.0703	0.0728
30	0.0359	0.0429	0.0660	0.0706
45	0.0253	0.0353	0.0581	0.0632
60	0.0150	0.0309	0.0512	0.0557
75	0.0155	0.0252	0.0500	0.0525
90	0.0125	0.0240	0.0331	0.0457

The method to determine kinetic data of folic acid and vitamin B_1 were the same as that described earlier in that of vitamins A and E. Tables 19 and 20 show the kinetic parameters obtained from the assumption of zero and first order kinetics. It can be observed on the basis of correlation coefficients (R) that vitamin B_1 followed zero order degradation reaction kinetic. For folic acid of goat milk tablets which kept in plastic bottles, the R values of first order reaction kinetic was better than of zero order reaction kinetic while both R values were practically identical for folic acid of goat milk tablets which kept in aluminium laminated pouches. So, it is not possible to

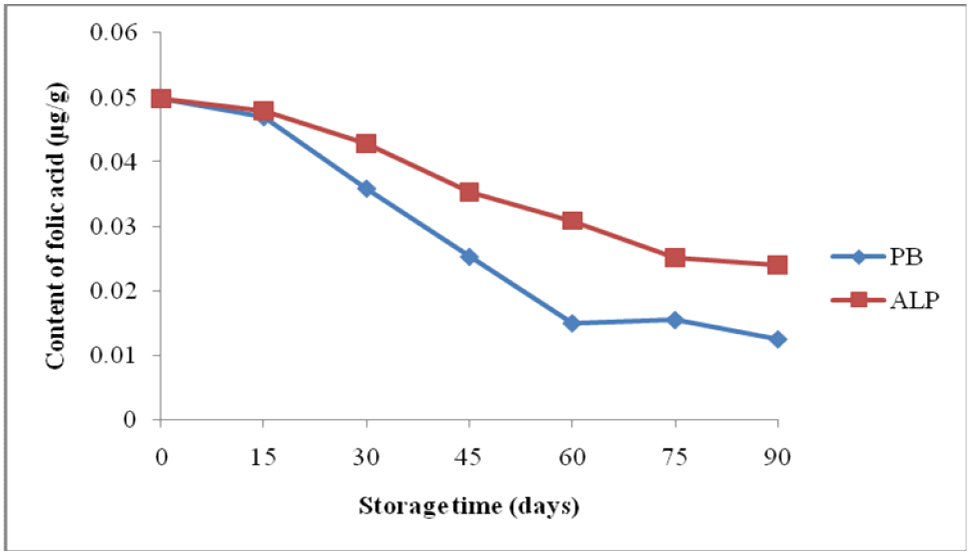
assure whether loss of folic acid in goat milk tablets followed a first or zero reaction kinetic, and it need longer period of storage time and more storage temperatures to know it.

Table 19 Non-linear regression analysis of folic acid and vitamin B_1 from zero order and first order reaction kinetics of goat milk tablets in plastic bottles

Parameter	Zero order			First order		
	K_0	C_0	R	K_1	C_0	R
Folic acid	-0.0019	0.1989	0.9327	-0.0172	-1.5247	0.9474
Vitamin B_1	-0.0018	0.3137	0.9594	-0.0084	-1.1163	0.9017

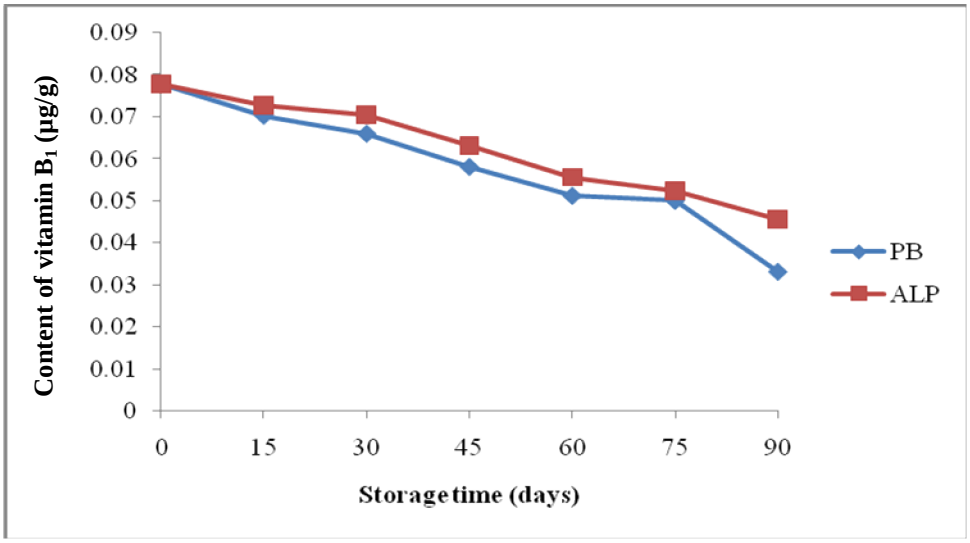
Table 20 Non-linear regression analysis of folic acid and vitamin B_1 from zero order and first order reaction kinetics of goat milk tablets in aluminium laminated pouches

Parameter	Zero order			First order		
	K_0	C_0	R	K_1	C_0	R
Folic acid	-0.0013	0.2038	0.9787	-0.0090	-1.5531	0.9760
Vitamin B_1	-0.0014	0.3157	0.9867	-0.0059	-1.1334	0.9761



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 12 Folic acid content of goat milk tablets during storage at accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches



PB = Plastic bottle, ALP = Aluminium laminated pouch

Figure 13 Vitamin B_1 content of goat milk tablets during storage at accelerated conditions (45°C and 90% relative humidity) in plastic bottles and aluminium laminated pouches

CHAPTER V
CONCLUSION

The purpose of this work was to produce goat milk tablets and to study the effect of plastic bottles and aluminium laminated pouches containers on nutritional compositions and stability of goat milk tablets. The nutritional compositions in this study consisted of major milk compositions (fat, protein, lactose and solid-not-fat), fat-soluble vitamins and water-soluble vitamins.

Pasteurized goat milk was spray dried. The milk powder obtained was use to prepare tablets by wet granulation method. The physical appearances of goat milk tablets prepared were canary yellow with shiny and smooth surface. The average weight of the tablet was 1.0394 ± 0.01 g. Its thickness and hardness were 4.497 ± 0.07 mm and 5.75 ± 0.67 kg, respectively.

The study of effect of plastic bottles and aluminium laminated pouches which stored at room temperature demonstrated that all of nutrients, fat, protein, lactose, solid-not-fat, vitamin A, E, B_1 and folic acid contents, showed no significant changed ($P > 0.05$).

Fat, protein, lactose, solid-not-fat contents in goat milk tablets which kept in plastic bottles and aluminium laminated pouches and stored at accelerated condition did not change while vitamin A, E, B_1 and folic acid contents decreased more in plastic bottles than in aluminium laminated pouches. From statistical analysis the results were not significantly different ($P>0.05$) for the effect of containers. The results in our study could be suggested that aluminium laminated pouches were better than plastic bottles in term of protection from vitamin A, E, B_1 and folic acid loss.

The changes of vitamin A, E and B_1 contents in goat milk tablets stored at accelerated condition followed zero order reaction kinetics. The kinetic constants for all vitamin changes in goat milk tablets stored in plastic bottles were more than those in aluminium laminated pouches. This suggests that aluminium laminated pouches are better than plastic bottles for storage of goat milk tablets. For folic acid content of goat

milk tablets, it needed extended time and higher storage temperatures to determine the order of reaction kinetic.

In this study, the analytical methods of vitamins A, E, B_1 and folic acid were rapid, accurate and reliable to use for routine analysis of wide variety of milk products with good accuracy, precision and reproducibility.

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APPENDICES

APPENDIX A

Method validation data

Table 21 Peak area of standard vitamin A at various concentrations

Injection no.	Concentration (µg/mL)				
	0.025	**0.05**	**0.1**	**0.5**	**1**
1	1192	2224	4598	22598	45820
2	1185	2259	4625	22610	45795
3	1189	2201	4639	22632	45808
Average	1188.67	2228	4620.67	22613.33	45807.67
SD	3.51	29.21	20.84	17.24	12.50
%RSD	0.30	1.31	0.45	0.08	0.03
Slope				45630	
***y*-intercept**				+51.31	
r^2				0.9999	
SD of slope				217.18	
SD of *y*-intercept				129.59	

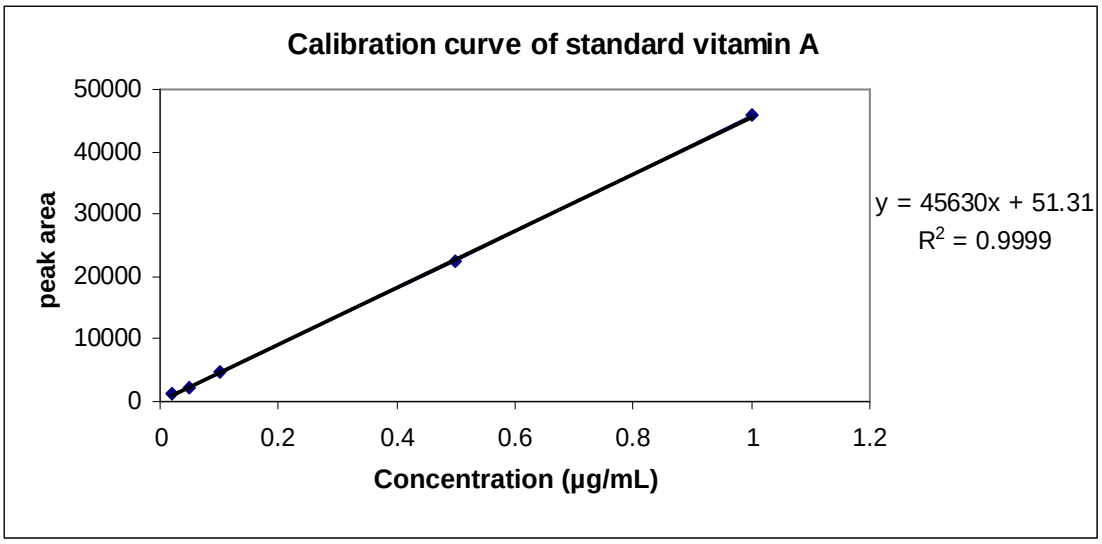


Figure 14 The calibration curve of standard vitamin A covering the range of 0.025-1 µg/mL

Table 22 Peak area of standard vitamin E at various concentrations

Injection no.	Concentration (µg/mL)				
	0.1	**0.2**	**0.5**	**1**	**2**
1	1211	2495	6739	14488	28808
2	1239	2528	6798	14511	28795
3	1238	2511	6807	14504	28811
Average	1229.33	2511.33	6781.33	14501	28804.67
SD	15.89	13.47	36.94	11.79	8.50
%RSD	1.30	0.54	0.54	0.08	0.03
Slope				14601	
***y*-intercept**				-331.09	
r^2				0.9998	
SD of slope				190.86	
SD of *y*-intercept				126.52	

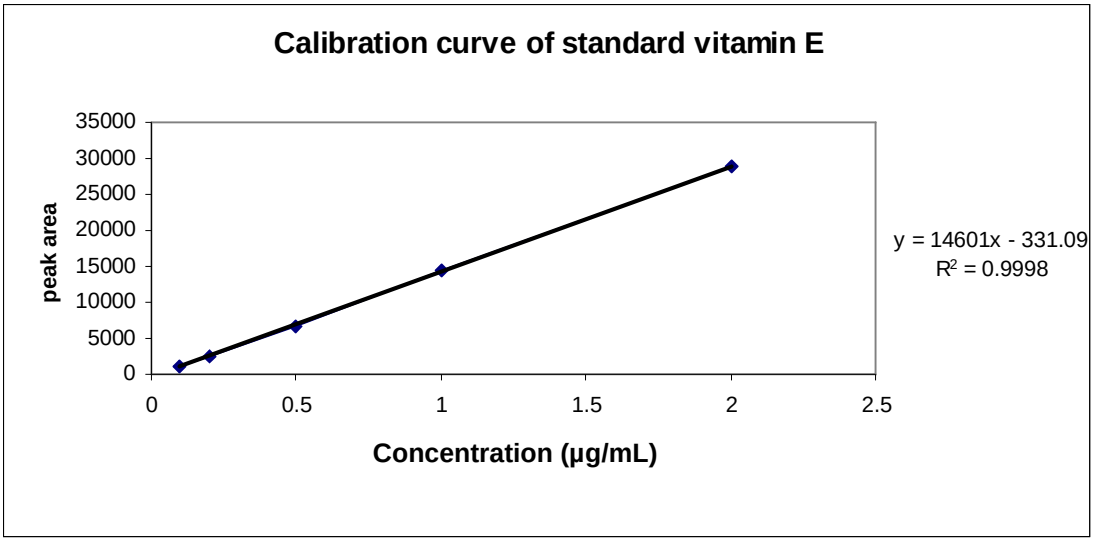


Figure 15 The calibration curve of standard vitamin E covering the range of 0.1-2 µg/mL

Table 23 Peak area of standard folic acid at various concentrations

Injection no.	Concentration (µg/mL)				
	0.05	**0.1**	**0.25**	**0.5**	**1**
1	2060	4040	11132	21988	43667
2	2048	4042	11216	22098	43875
3	2052	4057	11028	21997	43697
Average	2053.33	4046.33	11125.33	22027.67	43746.33
SD	6.11	9.29	94.18	61.07	112.43
%RSD	0.30	0.23	0.85	0.28	0.26
Slope				43962	
***y*-intercept**				-105.82	
r^2				0.9999	
SD of slope				226.79	
SD of *y*-intercept				150.35	

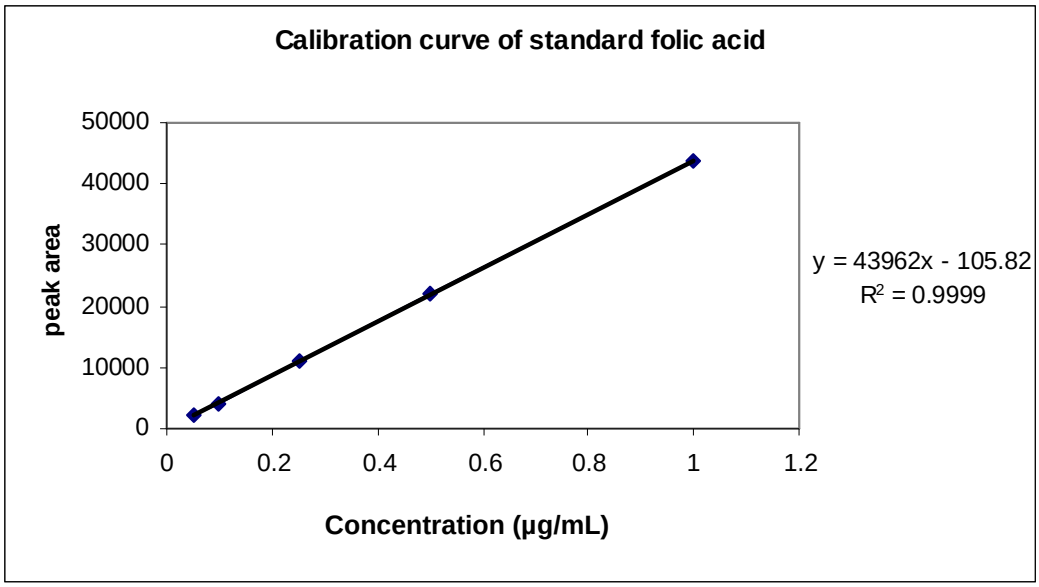


Figure 16 The calibration curve of standard folic acid covering the range of 0.05-1µg/mL

Table 24 Peak area of standard vitamin B_1 at various concentrations

Injection no.	Concentration (µg/mL)				
	0.1	**0.2**	**0.5**	**1**	**2**
1	2752	5666	15609	32853	64766
2	2738	5678	15557	32700	64977
3	2746	5594	15621	32890	64638
Average	2745.33	5646	15595.67	32814.33	64793.67
SD	7.02	45.43	34.02	100.73	171.19
%RSD	0.26	0.80	0.22	0.31	0.26
Slope				32832	
***y*-intercept**				-633.40	
r^2				0.9998	
SD of slope				432.70	
SD of *y*-intercept				286.85	

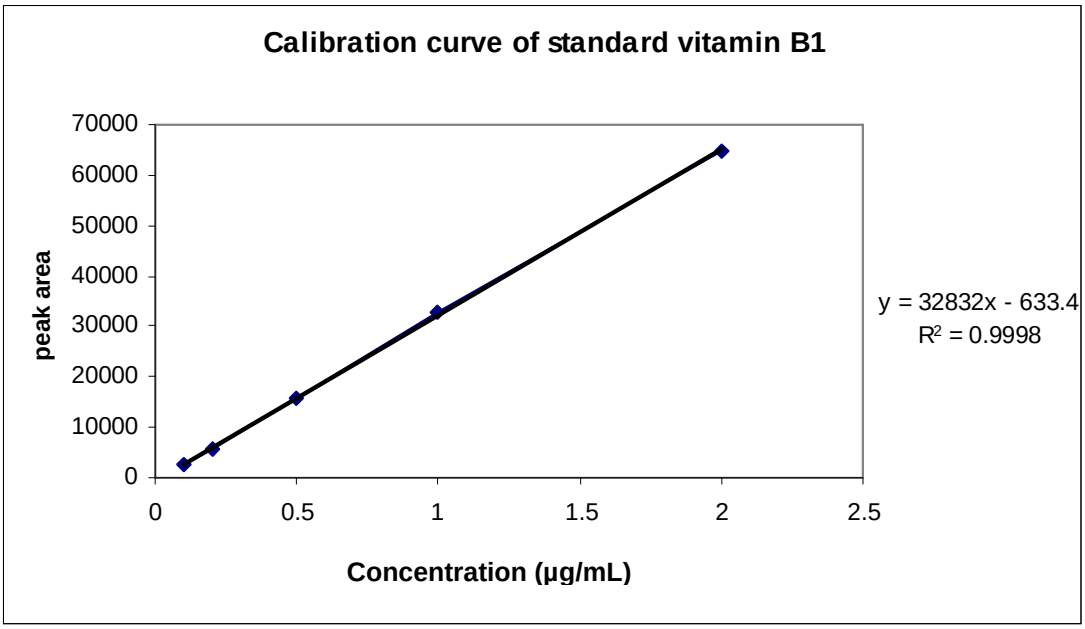


Figure 17 The calibration curve of standard vitamin B_1 covering the range of 0.1-2 µg/mL

Table 25 Analytical data of injection precision of vitamin A at 0.1 µg/mL (n=10)

No. of injection	t_R (min)	Peak area
1	5.31	6785
2	5.30	6755
3	5.31	6824
4	5.30	6786
5	5.31	6823
6	5.31	6757
7	5.31	6743
8	5.31	6797
9	5.31	6858
10	5.31	6770
Average	5.31	6789.80
SD	0.01	36.33
%RSD	0.08	0.54

Table 26 Analytical data of injection precision of vitamin E at 0.5 µg/mL (n=10)

No. of injection	t_R (min)	Peak area
1	10.99	4657
2	10.98	4776
3	10.98	4589
4	10.98	4553
5	10.98	4690
6	10.98	4723
7	10.98	4675
8	10.98	4624
9	10.99	4750
10	10.99	4640
Average	10.98	4667.70
SD	0.01	70.12
%RSD	0.04	1.50

Table 27 Analytical data of injection precision of folic acid at 0.25 µg/mL (n=10)

No. of injection	t_R (min)	Peak area
1	11.99	11132
2	12.02	11216
3	12.01	11028
4	11.99	11222
5	12.23	11098
6	12.11	11143
7	11.98	11220
8	12.00	11198
9	11.99	11207
10	12.01	11157
Average	12.02	11162.10
SD	0.08	63.72
%RSD	0.65	0.57

Table 28 Analytical data of injection precision of vitamin B_1 at 0.5 µg/mL (n=10)

No. of injection	t_R (min)	Peak area
1	14.12	15609
2	14.14	15557
3	14.12	15621
4	14.09	15544
5	14.12	15498
6	14.10	15333
7	14.10	15540
8	14.09	15670
9	14.08	15490
10	14.08	15552
Average	14.10	15541.40
SD	0.02	91.75
%RSD	0.14	0.59

Table 29 Analytical data of intra-day precision of vitamin A (n=6)

No. of injection	0.025 µg/mL		0.1 µg/mL		1 µg/mL	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	11.01	1190	10.95	4657	10.99	45778
2	11.00	1173	10.99	4776	11.01	45723
3	10.98	1180	10.98	4589	11.00	45832
4	10.99	1195	10.98	4553	10.98	45786
5	10.98	1165	10.99	4690	10.99	45865
6	11.02	1185	11.02	4723	11.00	45854
Average	10.99	1181.33	10.99	4664.67	10.99	45806.33
SD	0.01	11.08	0.02	83.30	0.01	53.91
%RSD	0.15	0.94	0.21	1.79	0.10	0.12

Table 30 Analytical data of intra-day precision of vitamin E (n=6)

No. of injection	0.1 µg/mL		0.5 µg/mL		2 µg/mL	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	5.29	1219	5.30	6785	5.28	28875
2	5.28	1280	5.30	6755	5.31	28791
3	5.29	1264	5.31	6824	5.30	28827
4	5.30	1259	5.29	6786	5.39	28826
5	5.29	1277	5.28	6823	5.29	28806
6	5.31	1272	5.28	6757	5.30	28754
Average	5.30	1261.83	5.29	6788.33	5.31	28813.17
SD	0.01	22.41	0.01	30.28	0.04	40.55
%RSD	0.20	1.78	0.23	0.45	0.75	0.14

Table 31 Analytical data of intra-day precision of folic acid (n=6)

No. of injection	0.05 µg/mL		0.25 µg/mL		1 µg/mL	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	11.94	2060	11.99	11132	12.02	43667
2	11.99	2048	12.02	11216	12.00	43875
3	11.98	2052	12.01	11028	11.97	43697
4	12.01	2047	11.99	11222	11.87	43703
5	11.99	2004	12.23	11098	11.99	43667
6	12.02	2062	12.11	11143	12.01	43769
Average	11.98	2045.50	12.06	11139.83	11.98	43729.67
SD	0.03	21.24	0.09	73.32	0.05	80.38
%RSD	0.23	1.04	0.79	0.66	0.46	0.18

Table 32 Analytical data of intra-day precision of vitamin B_1 (n=6)

No. of injection	0.1 µg/mL		0.5 µg/mL		2 µg/mL	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	11.01	2752	14.12	15609	10.99	64766
2	11.00	2738	14.14	15557	11.01	64977
3	10.98	2746	14.12	15621	11.00	64638
4	10.99	2741	14.09	15544	10.98	64731
5	10.98	2748	14.12	15498	10.99	64661
6	11.02	2728	14.10	15333	11.00	64686
Average	10.99	2742.17	14.12	15527	10.99	64743.17
SD	0.01	8.54	0.02	105.10	0.01	123.63
%RSD	0.15	0.31	0.12	0.68	0.10	0.19

Table 33 Analytical data of inter-day precision of vitamin A at 0.025 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	11.01	1190	11.01	1187	11.00	1189	10.98	1176	11.00	1223	11.01	1156
2	11.00	1173	10.99	1176	11.10	1209	10.99	1190	10.99	1176	11.00	1189
3	10.98	1180	10.98	1180	10.98	1197	10.98	1202	10.98	1198	10.98	1198
Average	11.00	1181	10.99	1181	11.02	1198.33	10.98	1189.33	10.99	1199	10.99	1181
SD	0.02	8.54	0.02	5.57	0.06	10.07	0.01	13.01	0.01	23.51	0.02	22.11
% RSD	0.14	0.72	0.14	0.47	0.58	0.84	0.05	1.09	0.09	1.96	0.14	1.87

Table 34 Analytical data of inter-day precision of vitamin A at 0.1 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	10.95	4657	10.97	4678	10.98	4690	11.00	4598	11.00	4665	11.02	4623
2	10.99	4776	10.99	4598	10.99	4558	10.99	4614	11.02	4590	10.99	4588
3	10.98	4690	10.98	4650	11.00	4532	10.98	4606	11.00	4587	10.98	4559
Average	10.97	4707.67	10.98	4642	10.99	4593.33	10.99	4606	11.00	4614	10.99	4590
SD	0.02	61.44	0.01	40.56	0.01	84.72	0.01	8.00	0.01	44.19	0.01	32.05
% RSD	0.19	1.31	0.09	0.87	0.09	1.84	0.09	0.17	0.10	0.96	0.09	0.70

Table 35 Analytical data of inter-day precision of vitamin A at 1 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	10.99	45778	10.99	45787	10.99	45980	10.99	45345	10.99	46767	11.00	46555
2	11.01	45723	11.01	45907	11.01	46321	11.01	45507	11.01	45534	11.01	46450
3	11.00	45832	11.03	45866	10.98	46211	11.00	45539	10.98	45897	11.03	47027
Average	11.00	45777.67	11.01	45853.33	10.99	46170.67	11.00	45463.67	10.99	46066	11.01	46677.33
SD	0.01	54.50	0.02	60.99	0.02	174.04	0.01	104.01	0.02	633.63	0.02	307.34
% RSD	0.09	0.12	0.18	0.13	0.14	0.38	0.10	0.23	0.14	1.38	0.14	0.66

Table 36 Analytical data of inter-day precision of vitamin E at 0.1 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	5.29	1277	5.30	1245	5.29	1249	5.31	1270	5.30	1228	5.31	1218
2	5.28	1280	5.31	1234	5.30	1239	5.31	1253	5.31	1259	5.31	1254
3	5.29	1264	5.30	1215	5.30	1217	5.30	1222	5.30	1277	5.29	1220
Average	5.29	1273.67	5.30	1231.33	5.30	1235	5.31	1248.33	5.30	1254.67	5.30	1230.67
SD	0.01	8.50	0.01	15.18	0.01	16.37	0.01	24.34	0.01	24.79	0.01	20.23
% RSD	0.11	0.67	0.11	1.23	0.11	1.33	0.11	1.95	0.11	1.98	0.22	1.64

Table 37 Analytical data of inter-day precision of vitamin E at 0.5 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	5.30	6785	5.29	6789	5.30	6850	5.31	6785	5.28	6775	5.30	6642
2	5.30	6755	5.30	6845	5.30	6886	5.30	6888	5.30	6837	5.30	6670
3	5.31	6824	5.29	6802	5.29	6739	5.29	6845	5.29	6707	5.29	6759
Average	5.30	6788	5.29	6812	5.30	6825	5.30	6839.33	5.29	6773	5.30	6690.33
SD	0.01	34.60	0.01	29.31	0.01	76.62	0.01	51.73	0.01	65.02	0.01	61.09
% RSD	0.11	0.51	0.11	0.43	0.11	1.12	0.19	0.76	0.19	0.96	0.11	0.91

Table 38 Analytical data of inter-day precision of vitamin E at 2 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	5.28	28875	5.29	28756	5.30	28645	5.31	28665	5.29	28775	5.29	28660
2	5.31	28791	5.29	28854	5.30	28766	5.30	28744	5.30	28691	5.29	28579
3	5.30	28827	5.30	28834	5.31	28709	5.31	28732	5.31	28676	5.31	28637
Average	5.30	28831	5.29	28814.67	5.30	28706.67	5.31	28713.67	5.30	28714	5.30	28625.33
SD	0.02	42.14	0.01	51.78	0.01	60.53	0.01	42.57	0.01	53.36	0.01	41.74
% RSD	0.29	0.15	0.11	0.18	0.11	0.21	0.11	0.15	0.19	0.19	0.22	0.15

Table 39 Analytical data of inter-day precision of folic acid at 0.05 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	11.94	2060	11.77	2078	11.65	2109	11.89	2060	11.87	2098	12.11	2101
2	11.99	2048	11.83	2085	11.73	2065	11.95	2099	11.99	2033	12.18	2045
3	11.98	2052	11.83	2069	11.76	2077	11.98	2115	12.05	2057	12.22	2080
Average	11.97	2053.33	11.81	2077.33	11.71	2083.67	11.94	2091.33	11.97	2062.67	12.17	2075.33
SD	0.03	6.11	0.04	8.02	0.06	22.74	0.05	28.29	0.09	32.87	0.05	28.29
% RSD	0.22	0.30	0.29	1.14	0.49	1.09	0.38	1.35	0.77	1.59	0.46	1.36

Table 40 Analytical data of inter-day precision of folic acid at 0.25 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	11.99	11132	11.86	11122	11.87	11156	11.81	11123	12.03	11122	12.21	11190
2	12.02	11216	11.85	11208	11.9	11224	11.78	11167	12.03	11221	12.19	11232
3	12.01	11028	11.86	11034	11.92	11179	11.75	11209	12.05	11199	12.19	11199
Average	12.00	11125.33	11.86	11121.33	11.90	11186.33	11.78	11166.33	12.04	11180.67	12.19	11207
								43.00	0.01	51.98	0.01	22.11
% RSD	0.13	0.85	0.05	0.13	0.21	0.31	0.25	0.39	0.10	0.46	0.09	0.19

Table 41 Analytical data of inter-day precision of folic acid at 1 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	12.02	43667	11.85	43689	11.95	43887	11.73	43698	12.02	43775	12.24	43788
2	12.00	43875	11.90	43707	11.96	43655	11.71	43776	12.02	43656	12.21	43676
3	11.97	43697	11.91	43658	11.99	43553	11.72	43880	12.01	43567	12.17	43939
Average	11.99	43746.33	11.88	43684.67	11.96	43698.33	11.72	43784.67	12.02	43666	12.20	43801
SD	0.03	112.43	0.03	24.79	0.02	171.16	0.01	91.30	0.01	104.36	0.03	131.98
% RSD	0.21	0.26	0.27	0.22	0.17	0.39	0.08	0.21	0.05	0.24	0.29	0.30

Table 42 Analytical data of inter-day precision of vitamin B_1 at 0.1 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	14.22	2752	14.03	2817	14.08	2767	13.87	2779	13.82	2732	13.96	2790
2	14.19	2738	14.08	2787	14.10	2856	13.96	2845	13.96	2812	14.06	2777
3	14.17	2746	14.11	2823	14.11	2798	14.02	2810	14.01	2770	14.10	2853
Average	14.19	2745.33	14.07	2809.00	14.10	2807	13.95	2811.33	13.93	2771.33	14.04	2806.66
								33.02	0.09	40.01	0.07	40.65
% RSD	0.18	0.26	0.29	0.60	0.11	1.61	0.54	1.17	0.70	1.44	0.51	1.45

Table 43 Analytical data of inter-day precision of vitamin B_1 at 0.5 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	14.12	15609	14.16	15667	14.04	15463	14.02	15489	14.05	15598	14.12	15590
2	14.14	15557	14.18	15632	14.02	15509	14.05	15565	14.06	15612	14.12	15478
3	14.12	15621	14.19	15703	14.01	15532	14.02	15534	14.05	15499	14.11	15530
Average	14.13	15595.67	14.18	15667.33	14.02	15501.33	14.03	15529.33	14.05	15569.67	14.12	15532.67
SD	0.01	34.02	0.02	35.50	0.02	35.13	0.02	38.21	0.01	61.60	0.01	56.04
% RSD	0.08	0.22	0.11	0.23	0.11	0.23	0.12	0.25	0.04	0.40	0.04	0.36

Table 44 Analytical data of inter-day precision of vitamin B_1 at 2 µg/mL (n=3)

No. of injection	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area	t_R (min)	Peak area
1	14.13	64766	14.26	64789	13.98	65009	14.00	65900	14.03	64798	14.17	65087
2	14.11	64977	14.23	65032	14.00	64703	14.00	66089	14.03	65009	13.98	64770
3	14.11	64638	14.23	64897	13.99	64718	14.02	65000	14.04	65012	14.09	64903
Average	14.12	64793.67	14.24	64906	13.99	64810	14.00	65663	14.03	64939.67	14.08	64920
SD	0.01	171.19	0.01	121.75	0.01	172.50	0.01	581.90	0.01	122.70	0.09	159.18
% RSD	0.08	0.26	0.12	0.18	0.07	0.27	0.08	0.89	0.04	0.19	0.68	0.25

Table 45 Recovery data of vitamin A from standard addition method in goat milk tablets sample (n=3)

Sample No.	Amount of standard vitamin A								
	added (µg/mL)	found (µg/mL)	% Recovery	added (µg/mL)	found (µg/mL)	% Recovery	Added (µg/mL)	found (µg/mL)	% Recovery
1	0.025	0.0208	83.20	0.1	0.0882	88.20	1	0.9054	90.54
2	0.025	0.0180	84.01	0.1	0.0901	90.10	1	0.9322	93.22
3	0.025	0.0215	86.00	0.1	0.0913	91.30	1	0.9268	92.68
Average			84.40			89.87			92.15
SD			1.44			1.56			1.40
%RSD			1.71			1.74			1.54

Table 46 Recovery data of vitamin E from standard addition method in goat milk tablets sample (n=3)

Sample No.	Amount of standard vitamin E								
	added (µg/mL)	found (µg/mL)	% Recovery	added (µg/mL)	found (µg/mL)	% Recovery	Added (µg/mL)	found (µg/mL)	% Recovery
1	0.1	0.0874	87.40	0.5	0.4592	91.84	2	1.8775	93.88
2	0.1	0.0901	90.10	0.5	0.4752	95.04	2	1.8990	94.95
3	0.1	0.0885	88.50	0.5	0.4667	93.34	2	1.9332	96.66
Average			88.67			93.41			95.16
SD			1.36			1.60			1.40
%RSD			1.53			1.71			1.47

Table 47 Recovery data of folic acid from standard addition method in goat milk tablets sample (n=3)

Sample No.	Amount of standard folic acid								
	added (µg/mL)	**found (µg/mL)**	**% Recovery**	**Added (µg/mL)**	**found (µg/mL)**	**% Recovery**	**Added (µg/mL)**	**found (µg/mL)**	**% Recovery**
1	0.05	0.0402	80.40	0.25	0.2232	89.28	1	0.9132	91.32
2	0.05	0.0398	79.60	0.25	0.2168	86.72	1	0.8995	89.95
3	0.05	0.0415	82.00	0.25	0.2202	88.08	1	0.8987	89.87
Average			80.67			88.02			90.38
SD			1.22			1.28			0.82
%RSD			1.51			1.46			0.90

Table 48 Recovery data of vitamin B_1 from standard addition method in goat milk tablets sample (n=3)

Sample No.	Amount of standard B_1								
	added (µg/mL)	**found (µg/mL)**	**% Recovery**	**Added (µg/mL)**	**found (µg/mL)**	**% Recovery**	**Added (µg/mL)**	**found (µg/mL)**	**% Recovery**
1	0.1	0.0906	90.06	0.5	0.4678	93.56	2	1.9355	96.78
2	0.1	0.0891	89.10	0.5	0.4605	92.10	2	1.9289	96.45
3	0.1	0.0886	88.60	0.5	0.4660	93.20	2	1.9196	95.98
Average			89.25			92.95			96.40
SD			0.74			0.76			0.40
%RSD			0.83			0.82			0.42

APPENDIX B

MilkoScan

A. Measuring Principle of MilkoScan.

Within the infrared spectrum, fat, protein, and lactose have characteristic absorption wavelengths. When the intensity of the light transmitted at precisely these wavelengths is measured, a measure of the concentration of the component in question is obtained. In practice, the absorption is also measured at another. “reference” wavelength close by, and the two values are subtracted from each other to remove of milk.

In order to ensure that only the light of the desired wavelength is detected, optical filters (interference filters) which permit only the desired wavelength to pass are employed. In MilkoScan, which rotates to bring each of the filters in turn – 2 per component – into position in the path of the infrared beam.

Although the wavelength are carefully chosen, each component absorbs a small amount of infrared energy at the wavelength of the others, e.g. although the fat filters mainly detect the presence of fat, small amount of protein and lactose are also registered by them. Therefore the signals received from each pair of filters will not give a completely accurate value for the concentration of the component concernd. This problem is solved mathematically in the micro-processor by adding to the fat filters signals a certain percentage of the reading obtained by the protein and lactose filters pairs. The other combinations are similarly compensated for. These corrections are called “intercorrection factors”.

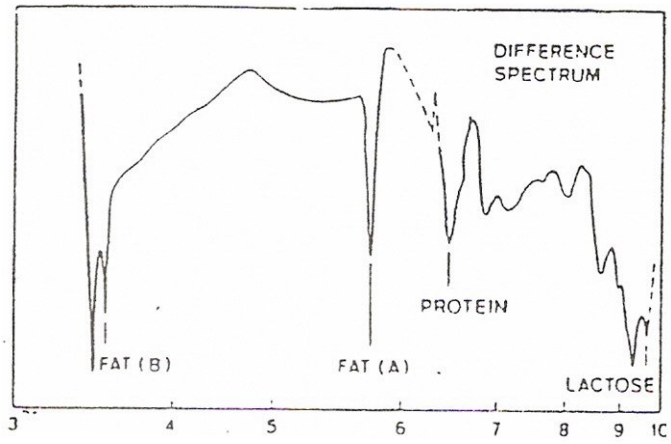


Figure 18 IR-spectrum of milk versus water illustrating the characteristic absorption peaks from fat, protein, and lactose

B. Fat Determination

1. Standard methods of analysis

The traditional standard methods for fat analysis are based either on weight or volumetric determination. They involve destruction of the globule structure of the butterfat, separation and then either drying and weighing of the fat or reading off the amount on a calibrated tube.

The most commonly used methods are:

-Röse-Gottlieb

-Schmid-Bondzynski-Ratzlaff (SBR)

-Mojonnier

-Gerber

-Babcock

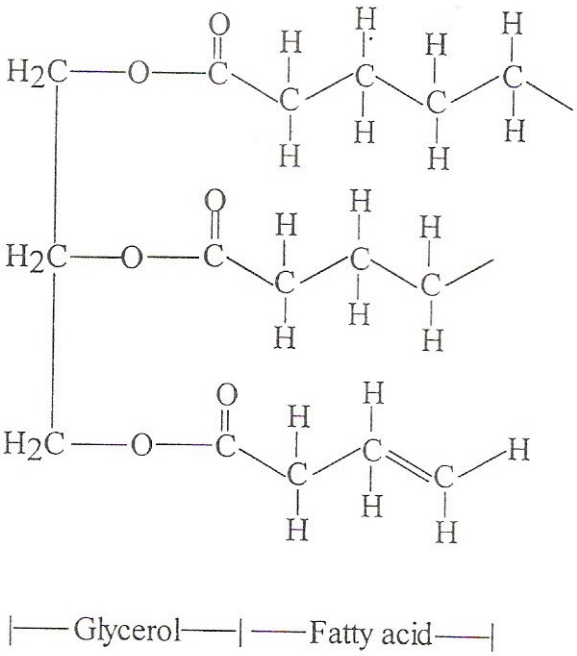
The first three are the most accurate, but Gerber and Babcock are much faster and most widely used.

An important difference between Röse-Gottlieb and Mojonnier on one hand and SBR, Gerber, and Babcock on the other hand, is that the first two will not include all free fatty acids formed in the samples. Old samples or samples which have

been exposed to vigorous mechanical treatment resulting in formation of free fatty acids will consequently by measured with lower results by the Röse-Gottlieb test than with for instance a Gerber test.

2. Measuring principle

The fat molecule consists of a glycerol “backbone” to which three fatty acid chains are bound:



Two different wavelengths, 5.7 µm and 3.5 µm, can be used to determine the fat in milk. The 5.7 µm filter is referred to as fat A, 3.5 µm as fat B.

2.1 Fat A

The absorption at 5.7 µm is due to stretching vibrations in the C=0 bounds of the carbonyl group in fat, and this measurement “counts” the number of fat molecules regardless of the length and weight of the length and weight of the individual fatty acids.

If the average chain length (mean molecular weight) of the fatty acid is changed, the number of triglyceride molecules per unit weight will change too, and an error will occur in the results unless the change is compensated for by recalibration the instrument. The composition of butterfat varies with season, region, breed, cow, and stage of lactation, and this means that an instrument using the 5.7 µm filter must be recalibrated when, for instance, going from winter to summer.

2.2 Fat B

The absorption at 3.5 µm is due to stretching vibrations in the saturated C-H bonds of the fatty acids chains. This measurement is, related to both the size and the number of fat molecules in the sample, as the number of carbonhydrogen bonds increase substantially in proportion to the molecular size. Both –CH3 and –CH2 groups absorb infrared energy at 3.5 µm, but the C-H stretching is markedly reduced by the presence of double bonds adjacent to these groups. The absorption decreases as a function of the degree of unsaturation (the number of –C=C- bonds), but even so, the 3.5 µm determination is less sensitive to variation index in cows milk than the 5.7 µm determination is, because it reflects the variation in chain lengths.

Another advantage of the 3.5 µm wavelength is that the measurement includes free fatty acids that may have formed during storage; these be measured at 5.7 µm.

Protein and lactose contribute to be the absorption at 3.5 µm, but their interference is removed by means of suitable intercorrection factors in the equation that calculate the fat content from the instrument signals.

2.3 The difference of fat A and fat B.

The effect of seasonal variations can be illustrated by the following example, which reflects the typical change in Danish bulk milk when going from winter to summer.

	Winter	Summer
Refractive index (n_D, 40 ^{0}C)	1.4530	1.4560
Iodine value (reflects the Number of C=C bonds)	28	43

If the refractive index changes from the lowest to the highest value the corresponding effect on the results at approx. 4% level will be :

MilkScan Fat A -0.24% fat

MilkScan Fat B -0.05% fat

The difference between results from the two wavelengths is in many cases negligible, but analyses of all kinds of milk-especially individual cow's milk should be performed using the 3.5 μm filter for optimal performance.

On the other hand products, which contain large amounts of sugars, like ice cream or sweetened condensed milk, should preferably be measured fat A, due to the large intercorrections from sugar to fat B. Fat A will also be superior to fat B for products containing vegetable fat, where the degree of saturation (iodine value) may very greatly.

C. Protein Determination

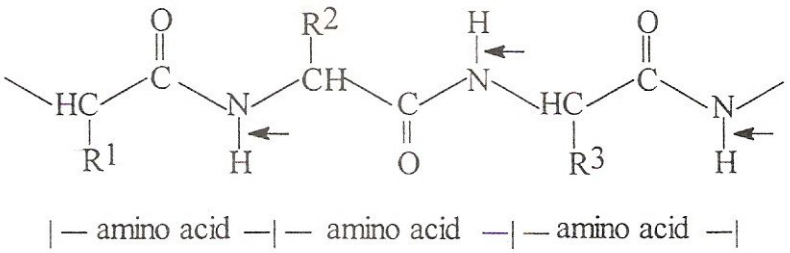
1. Standard method of analysis

The traditional standard method for protein analysis is the Kjeldahl method, which is also the most accurate. It is, however, based on a determination of the total nitrogen content of the sample, and detects not only the nitrogen in protein molecules, but also non-protein nitrogen, with the exception of nitrates and some nitrites. Through heating in concentrated sulphuric acids, the nitrogen is converted to ammonium sulphate. After distillation in an alkaline medium, the amount if nitrogen can be determined by an ammonia titration.

Another commonly used standard method for protein is the dye-binding solution, which exploits the capacity of the basic amino acid groups in the protein molecule to bind the dyestuff amino black. Thus it is actually the number of basic amino acid groups which are counted. The complex formed at a stabilized pH by the protein and amino black is separated out and than a photometric measurement of the remaining amount of dye is made. The method correlates well with Kjeldahl, and since it is considerable faster, it is often used, especially within the dairy industry.

2. Measuring principle

The molecule consist primarily of amino acid units joined together in a long chain by peptide bonds :



The wavelength for protein determination is 6.5 µm, and it is the nitrogenhydrogen bonds within the peptide bonds that are responsible for the IR absorption. Thus, the measurement represents the number of amino acids rather than their weight, but as the composition of protein in milk is fairly constant, this causes, on problems. In contrast to the Kjeldahl analysis, which is the reference method, the infrared measurement dose not includes non-protein-nitrogen.

D. Lactose Determination

1. Standard methods of analysis

The most commonly used standard method for the determination of lactose is Polaimetry method, which is base on the optical rotation of polarized light. Carbohydrates (including sugars such as lactose) rotate polarized light to different, specific angles. The lactose is first separated out and filtered, after which it is subjected to a beam of polarized light, the angle of rotation is measured, and the amount of lactose present calculated.

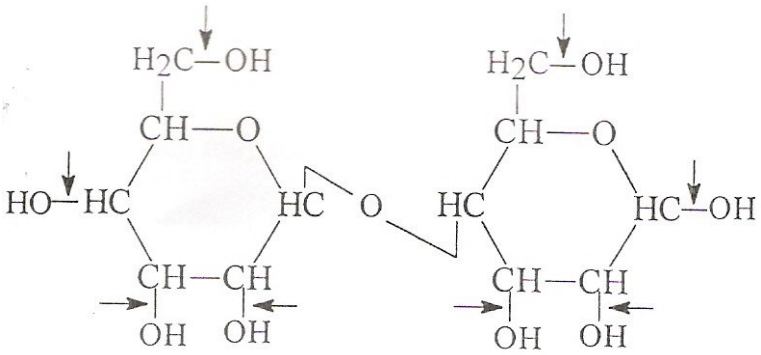
A second method of lactose analysis is IDF 79, an enzymatic method, which is specific for lactose. It is based on the enzymatic breakdown of molecules into glucose and galactose.

Finally, some carbohydrates, including lactose, can be measured by exploiting their ability to reduce other reagents, which are determination either

gravimetrically. This method has in common with Polarimatry that it can only be used to measure one, known compound at a time.

2. Measuring Principle

Carbohydrate molecules are composed of various combinations of carbon, hydrogen, and oxygen atoms, and may be built up of smaller units. For example, lactose consists of a glucose molecule and a galactose molecule joined together:



The hydroxyl group (OH) is characteristic of carbohydrates and it is the bond between the hydroxyl group and the carbon atom which absorbs IR energy at the lactose wavelength, 9.5 μm.

From the above it will be clear that the MilkoScan's "lactose" determination is not actually specific for lactose, but will also include other carbohydrates which may be present in the sample.

E. Total solid (TS) and Solid not fat (SNF) Determinations

Total solid or solid not fat are computed by assigning experimentally determined factor to percentage of all other solid milk components, and by adding this amount to appropriate % fat, protein, and lactose. Latter method has been shown to be more accurate method of determining milk solids.

F. Factors affection the infrared results

The MilkoScan employs as mentioned earlier an indirect measuring principle, which correlates absorption from certain chemical bonds to actual component concentrations.

The factors which may influence the IR measurement and consequently cause the need for individual calibration are as follows:

1. Changes in mean molecular weight
2. Homogenization
3. Sample temperature
4. Heat treatment
5. Lipolysis
6. Lactic acid
7. pH
8. Salt (NaCl) and other minerals
9. Addition of non-dairy fat
10. Additive (emulsifiers and stabilizers)
11. Sugars (other carbohydrates)
12. Preservatives

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