T.C.
KAHRAMANMARAS SUTCU IMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

MINOR COMPONENTS OF WHEY BUTTER

ARAM MAHDI MOHAMMED

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

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MINOR COMPONENTS OF WHEY BUTTER

(M.Sc. THESIS)

ARAM MAHDI MOHAMMED

ABSTRACT

The present study was conducted to produce butters from whey (W) obtained from four different collection centers (Uşak, Adana, Burdur ve Konya) in Turkey, and to determine their minor components namely total protein, total carotenoids, total chlorophyll, tocopherols (α-, β- γ- and total) and minerals (Ca, P, Mg, K and Na). The obtained results were compared with the similar properties of two commercially produced milk butters.

In general, the amounts of minor component were higher in the milk butters than the whey butters. However, a small difference was observed in terms of total tocopherol and distribution of tocopherol isomers, and the whey butters were high in chlorophyll content. The observed difference between the milk and whey butters suggests that milk and whey used for the butter production significantly differ in the chemical composition. There were also variations in the minor components of the whey butters. Type of cheese produced and the methods used for cheese manufacturing in each city of the collection centers were definitely the main sources that affected the final composition of whey. Moreover, the Na concentration was found high in two whey butters (Burdur and Konya), probably the result of salting or brine solution during cheese manufacturing, in which NaCl passes through whey.

In conclusion, this study shows the significance of evaluating the minor components of butter. The obtained results would be the source of data for creating a codex for whey butter because the number of whey collection centers, as potential whey producers, increases today.

Keywords: Whey, Butter, Protein, Carotenoid, Chlorophyll, Tocopherol, Minerals

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PEYNİRALTI SUYU TEREYAĞINDAKİ MİNÖR BİLEŞİKLER
(YÜKSEK LİSANS TEZİ)
ARAM MAHDI MOHAMMED
ÖZET

Bu çalışmada, endüstriyel bir atık olan ve dört farklı ildeki toplama merkezlerinden (Uşak, Adana, Burdur ve Konya) sağlanan peyniraltı sularından (PAS) tereyağları üretilmiş ve tereyağların minör bileşiklerinden olan toplam protein, toplam karotenoid ve toplam klorofil, tokoferol (α-, β- γ- ve toplam) ve mineral maddeler (Ca, P, Mg, K ve Na) araştırılmıştır. Elde edilen sonuçlar ticari olarak üretilen iki süt tereyağlarının benzer özellikleri ile karşılaştırılmıştır.

Minör bileşiklerin miktarı, genel olarak, süt tereyağlarında daha fazla bulunmuştur. Ancak, tokoferol izomerlerinin dağılımının ve toplam tokoferol miktarının her iki tereyağında da birbirlerine yakın, klorofil miktarının ise PAS tereyağlarında daha fazla olduğu belirlenmiştir. Her iki tereyağı türünde görülen bu farklılıklar, kimyasal kompozisyonları farklı iki kaynağın, süt ve PAS'ın, kullanılmasında dolayı oluşmuştur. PAS tereyağları arasında minör bileşiklerin dağılımı bakımından farklılıklar tespit edilmiştir. Bu farklılıkların, toplama merkezlerinin bulunduğu illerde üretilen peynir çeşidinden ve uygulanan peynir üretim metodundan kaynaklandığı ve sonuçta ortaya çıkan PAS'ın kimyasal kompozisyonu etkilediği belirlenmiştir. Ayrıca, iki PAS tereyağında (Burdur ve Konya), Na konsantrasyonu yüksek çıkmış olup, muhtemelen peynirlerin kuru tuzlama veya salamura işlemlerine tabi tutulmaları sonucu tuzun PAS'a geçmelerinden ileri gelmiştir.

Sonuç olarak, bu çalışma, tereyağlarında minör bileşiklerin belirlenmesinin önemli olduğunu göstermiştir. Elde edilen bu sonuçlar, günümüzde hızla sayısı artan PAS toplama merkezlerinde muhtemel üretilecek olan PAS tereyağları için hazırlanacak kodeks çalışmalarına veri sağlamada kaynak oluşturacaktır.

Anahtar Kelimeler: Peyniraltı suyu, Tereyağı, Protein, Karotenoid, Klorofil, Tokoferol, Mineral maddeler

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1. INTRODUCTION

Food waste is characterized by the unavoidably generation of specific by-products during processing. Waste disposal in food industry is of primary concern to environment and sustainability since most food waste contains organic and inorganic residues.

In response to the growing interest for cheap and valuable food components, food by-products are considered as excellent sources of many components that possess functional and nutritional properties. Today, efficient use of by-products provides substantial economic gains for the countries and significant reduction in environmental pollution. Nowadays, the existent technologies allow the recovery of target compounds from the by-products and their recycling inside food chain as foods or functional additives in different products.

Whey is the remaining liquid from the precipitation and removal of milk casein during cheese making. Whey is the main by-product of cheese making, and represents about 85-95% of the milk volume and retains 55% of milk nutrients (de Wit, 2001). It contains serum proteins, lactose, fats, mineral salts, and vitamins at different extent. Its composition varies considerably in relation to the milk and processes used for cheese making (Akyüz, 1979; Kurt, 1981; Kurt and Gülümser, 1988; Yalçın *et al.*, 1994).

In the past, whey was considered as a waste product, and disposed on to farmland or into waterways, or only as useful, in part, for animal feeding. However, whey dumping causes serious environmental pollution, having prompted governments and regulatory authorities to ban the disposal of untreated whey. Biological and chemical oxygen demand (BOD and COD) of whey have been reported to be 30-50 gL^{-1} and 60-80 gL^{-1}, respectively (Siso, 1996; Smithers, 2008; Božanić *et al.*, 2014; Yadav *et al.*, 2015). The fat and lactose content are the most important factors that cause these BOD and COD values (Carvalho *et al.*, 2013). These organic substances disrupt ecological balance because they use oxygen in soil and water; promote the growth of toxic algae and plant species and threaten human and animal health.

Nowadays advances in processing technologies allow whey to be transformed into value-added products. Whey is treated or transformed via drying, concentration, filtration or fermentation to whey products i.e. whey powder, whey protein concentrate and isolate, lactose, casein and fat. Besides high nutritional value and health benefits, these products have several functional properties useful for water binding, gelation, thickening,

emulsification, foaming, whipping, taste and flavor, and find practical applications in infant foods, candies, soups, sauces, soft drinks, and processed meat, dairy and bakery products.

According to the Turkish Statistical Agency (TÜİK, 2017), 17-20% of raw milk produced in Turkey was used for cheese production, and only 500 KT of the produced whey is utilized to the products as liquid, concentrated and powder forms, and lactose. The amount of unutilized whey, roughly 2.5-3.0 MT per year, is discarded without any economic advantage. The butter production in Turkey was 52 KT in 2017. Since it was insufficient to meet domestic consumption, the agency reported that around 22.5 KT butter was imported annually. When considering the amounts of whey produced in Turkey, it is possible to produce appreciable amounts of butter.

2. SUBJECT, SCOPE AND PURPOSE OF THE RESEARCH

In the literature search made in line with the possibilities, there was no information about the production and use of whey butter in Turkey. In addition, whey butter has not been studied extensively up to now. Therefore, whey butter was chosen as the subject in this research.

This research was complementary to three previous researches completed on the butters made from whey obtained from different whey collection centers around Turkey: (I) basic characteristics, (ii) thermal and oxidative properties, and (iii) volatiles.

This research aimed to determine and compare the amounts of minor components of the whey butters produced from the whey obtained from the collection centers in four cities (Uşak, Adana, Burdur and Konya) in Turkey. In order to achieve this goal, it was necessary to investigate the contents of proteins, carotenoids, chlorophylls, tocopherol isomers and minerals in the whey butters, and to compare the obtained data with those from the commercial milk butters.

3. LITERATURE REVIEW

In parallel with the increase in population, the number of food producing enterprises in the world is also increasing. There is no doubt that this would increase the amount of food waste and, consequently, waste problems. Food processing byproducts are waste in solid or liquid form containing high amounts of organic compounds. For this reason, effective assessment of this waste is important in terms of prevention of environmental pollution and protection of ecological balance.

Today, food by-products have been considered as cheap sources of nutritional and functional components, and as important food supplies to rapidly increasing world population. The developing technologies provide the recycling of the targeted components found applications in the food chain as nutrients or functional additives (Galanakis, 2012).

The intensive and large-scale production of milk and milk products has generated huge amounts of whey and buttermilk, and an enormous disposal problem for the dairy industry. These by-products are primarily whey and butter milk, and contain a variety of readily biodegradable organic compounds i.e. lactose, fats and water-soluble proteins.

3.1. Whey

Whey is a green-yellowish liquid obtained after the precipitation and removal of milk casein in cheese making processes. About 9 L of whey is generated for every kilogram of cheese manufactured. Milk components that are polar or charged in nature such as lactose, whey proteins, and most minerals generally remain dispersed in the water phase during cheese making. Many of these compounds are therefore partitioned into whey (Table 3.1), i.e. fat and casein proteins aggregate into a curd whereas soluble whey proteins, lactose and minerals are drawn off in the whey (Beliveau, 2012).

Differences in milk quality and cheese making processes result in variations in the composition and sensory properties of whey. In general, whey represents 85-95% of the volume and 50% of the solids of milk used in the cheese making. The solids in whey (6-7%) are comprised of about 4.5-6.0% lactose, 0.6-0.8% water soluble proteins, 0.3-0.5% lipid and 0.8-10% (in dry matter) mineral salts (Ben-Hassan and Ghaly, 1994; Yalçın *et al.*, 1994; Siso, 1996; Smithers, 2008; Yadav *et al.*, 2015). Whey also contains appreciable amounts of lactic acid, non-protein nitrogen compounds, and vitamin B (Siso, 1996;

Bonnaillie and Tomasula, 2008; Smithers, 2008; Dragone *et al.*, 2009; Yadav *et al.*, 2015).

Whey is classified into two groups based on the production process. Sweet whey results from the manufacture of hard cheeses, e.g., Cheddar, Mozzarella, Swiss, Brick, and Gouda, whereby the coagulum is formed by enzymatic (rennet) coagulation at minimum pH 5.6. Acid whey is produced from the manufacture of fresh cheeses, e.g., Cottage, Cream, Ricotta and Chevre, where the coagulum is achieved at maximum pH 5.6 by acidification. Compare to sweet whey, acid whey have higher levels of acidity and ash with Ca and P (Table 3.2) (Siso, 1996; Abd El-Salam *et al.*, 2009; Tsakali *et al.*, 2010). The composition of whey varies depending on animal species, breeding, the stage of lactation, forage, season, milk composition, cheese processing and whey type (Tsakali *et al.*, 2010).

Table 3.1. Selective concentration of milk component during cheese manufacturing

Components	**% Lost to whey**	**Components**	**% Lost to whey**
Water	95.5	Whey protein	92.9
Lactose	96.0	Casein	4.0
Fat	7.5	Salts	50.0

(Beliveau, 2012)

Table 3.2. Chemical composition of whey on liquid basis

	Milk	**Sweet whey**	**Acid whey**
Water (%)	85.5-90.6	93.0-94.0	94.0-95.0
pH	6.5-6.7	5.6-6.4	3.6-5.6
Total solids (%)	10.5-14.5	6.0-6.8	5.0-6.8
Protein (%)	2.3-4.4	0.6-1.0	0.8-1.0
Fat (%)	2.5-4.0	0.3-0.5	0.1-0.4
Lactose (%)	3.6-5.5	4.5-5.1	3.8-4.4
Ca (mg/L)	800-2000	349-365	900-950
P (mg/L)	890-1580	430-510	550-580
Mg (mg/L)	80-220	63-65	80-90
K (mg/L)	1096-2326	1230-1400	1500-1530
Na (mg/L)	320-594	395-455	390-400

(Wong *et al.*, 1978; Early, 1998; de Wit, 2002; Huppertz *et al.*, 2006; Walstra *et al.*, 2006; Tsakali *et al.*, 2010; Smithers, 2015; Yadav *et al.*, 2015)

3.2. Whey Products

Over the last few decades, dairy companies have applied different technologies to process cheese whey resulting in its separation into its principle components, comprising fractions enriched in proteins, lactose and minerals (Figure 3.1) (Mollea *et al.*, 2013). These technologies have been generally based around crystallization, membrane and chromatographic processes. The separation of whey components allows use them in variety of food products for specific purposes, e.g., emulsification, gelation, foaming, solubility, water- and fat-holding capacity and nutritional value (Casper *et al.*, 1999; de Wit, 2001; Walstra *et al.*, 2006; Mollea *et al.*, 2013) (Figure 3.2).

3.2.1. Liquid, condensed and powdered cheese whey

Concentration and drying is the simplest operation used in whey utilization. Typical traditional operations consist of evaporation in multistage vacuum evaporators, and/or followed by spray or roll drying. These forms of whey are, in general, produced from sweet whey, and maintained for a longer period of time, facilitating storage and transportation (Kosikowski, 1979).

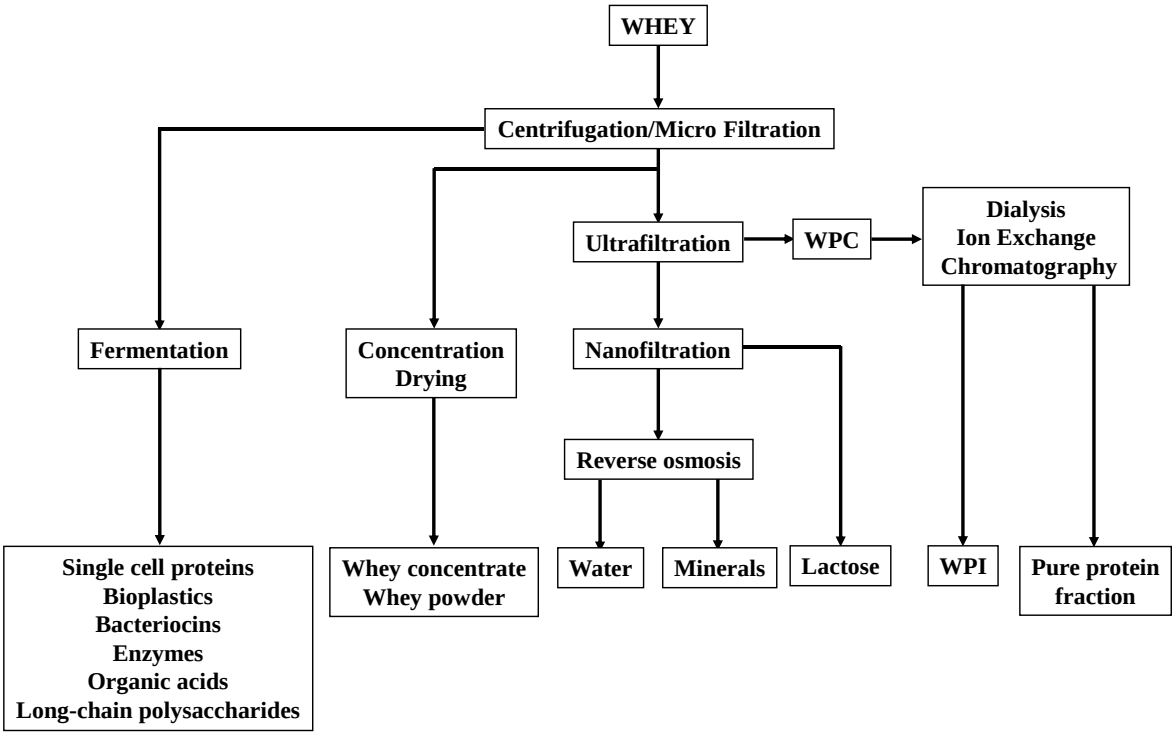

Figure 3.1. Products of whey processing

Whey Products	Whey	Whey Concentrated/Powdered					Whey Protein Concentrated/Powdered			Lactose	
	Liquid	*Raw*	*Sweetened*	*Demineralized*	*Deproteinized*	*Delactosed*	*Demineralized*	*Delactosed*	*Demineralized and Delactosed*	*Raw*	*Refined*
Animal feeds	X	X		X	X	X					
Human foods											
Baby foods				X			X	X	X		X
Diet foods				X			X	X	X	X	X
Meat products				X			X				
Soups		X	X	X							
Bakery products	X	X		X			X				
Salad dressing		X		X							
Cheese		X		X							
Beverages	X								X		
Confectionaries		X	X	X							X
Yeast production	X										

Figure 3.2. Whey and whey products used in foods

The principal market for liquid whey is animal feeding, but smaller quantities have been found applications in food industry after clarification and pasteurization (dairy products, bakery products, meat products, confectionary, beverages, infant foods, soups etc.).

Factors such as excessive saline taste and problems related to high lactose content (hygroscopicity, lumping, caking and browning) interfere with the use of whey powders in food products. However, recent technologies allow the production of demineralized, delactosed, deproteinized and defatted or fat-enriched whey concentrate (40-62% dry matter) or powders (5-6% moisture). These products are added to fruit juices, sport and carbonated beverages, dairy products (cheese, yogurt, milk shake and ice cream), bakery products (bread, cakes and biscuits), infant foods, soups, chocolate, coffee cream and souses for the replacement of defatted milk and milk powder. They balance aroma and sweetness, increase nutritional value, decrease calorie and prevent lactose crystallization and texture defects (Kosikowski, 1979; Siso, 1996; Early, 1998; Beckett, 2002; de Wit, 2002; Kelly, 2002; Mahoney, 2002).

“Rivella”, a bitter-sweet carbonated beverages, has been produced from liquid or concentrated whey in Sweden and Canada since 1950, because their aroma profile resemble citrus aroma (de Wit, 2002).

Whey powder plays important role together with emulsifiers in bakery products to improve aroma, crust color, loaf volume, texture and keeping quality (de Wit, 2001; Smithers, 2008; Mete, 2012).

3.2.2. Whey protein products

Whey protein comprises the group of milk proteins, and corresponds to about 18–20% of the total milk proteins. The major protein components of whey include α-lactalbumin (α-LA), β-lactoglobulin (β-LG), bovine serum albumin (BSA), and immunoglobulins (Ig), representing, 50%, 20%, 10% and 10% of the whey fraction, respectively. Besides, whey contains minor proteins such as lactoferrin (LF), transferrin (TF), lactoperoxidase (LPO), proteose peptone (PP), osteopontin (OPN) and lysozyme (LZ) (Huppertz *et al.*, 2006; Abd El-Salam *et al.*, 2009; Buffoni *et al.*, 2011; Santos *et al.*, 2012; Kukovics and Németh, 2013; Mollea *et al.*, 2013; Yadav *et al.*, 2015).

Whey proteins are globular proteins with a limited number of disulfide bonds, conferring a certain degree of structural constraint and impart stability. Whey proteins are more heat-sensitive, less calcium-sensitive, and can engage in thiol-disulfide interchanges to form oligomeric structures than milk casein (Kinsella and Whitehead, 1989)

Several studies have been reported that milk proteins possess important health benefits such as the transport of retinol, palmitate, fatty acids, vitamin D and cholesterol, the induction of apoptosis in tumor cells, the prevention of cancer, the host defense against organisms requiring iron, the antimicrobial and antiviral activity and the antiviral activity against HIV (Santos *et al.*, 2011; Mollea *et al.*, 2013). Also, whey proteins have higher PER (protein efficiency ratio), and contain essential, sulphur-containing and branched-chain amino acids (Siso, 1996; Ha and Zemel, 2003; Smithers, 2008; Yadav *et al.*, 2015). In this regard, whey protein products are useful for feeding of babies, elderlies, patients and athletes (Regester *et al.*, 1992; Smithers *et al.*, 1996; Fox, 2002; Walzem *et al.*, 2002; Smithers, 2008).

Whey protein products are excellent food ingredients in GRAS (Generally Recognized as Safe) status, and widely used in food industry, because of their unique functional characteristics (Jayaprakasha and Yoon, 2005; Abd El-Salam *et al.*, 2009). These characteristics are related to their interaction with water, proteins, carbohydrate, lipids and air. Eventually, whey proteins possess the abilities of forming viscosity, texture, emulsification, gelation and foam, and binding water (Table 3.3). However, these abilities

strictly depend on the amino acid composition and sequence, conformation, shape, size, flexibility, net charge, molecular hydrophobicity, denaturation degree of whey protein molecules. The production methods of WPI (e.g. isolation, purification and drying) and the properties of foods (e.g. chemical composition pH and ionic strength) where WPI is used also largely determine the functional properties of WPI (Kinsella and Whitehead, 1989; Foegeding and Luck, 2002; Walzem *et al.*, 2002; Dec and Chojnowski, 2006; Abd El-Salam *et al.*, 2009).

Table 3.3. Functional properties of whey and milk proteins

	Whey Proteins		**Milk Protein**
Functions	**WPC**	**WPI**	**Concentrate**
Solubility	xxx	xxx	xx
Emulsification	xx	xx	x
Foaming	xx	xx	x
Water binding	x	x	x
Viscosity	x	x	x
Jelling	xxx	x	---
Heat stability	x	x	x
Acid stability	xxx	xxx	x
Freeze-thaw stability	x	x	x

x, poor; xx, good; xxx, excellent.
(Singh, 2002)

Worldwide production of individual whey proteins represents a challenge to food technologist to find interesting ways for its utilization. Developments in industrial membrane separation including microfiltration, ultrafiltration, nanofiltration and reverse osmosis allow the production of whey protein products showing more uniform composition and consistent functional behavior in foods.

Whey protein products available in markets are concentrates (WPC), isolates (WPI), protein fractions (α-LA, β-LG, LF, LPO and casein glycomacropeptide) and hydrolysates (WPH) (Bonnaillie and Tomasula, 2008; Abd El-Salam *et al.*, 2009; Santos *et al.*, 2012).

3.2.2.1. Whey protein concentrates (WPC)

Ultrafiltration is the most commonly method used in the manufacture of WPC. It has been alone or in a combination with other processes such microfiltration, nanofiltration electrodialysis, reverse osmosis or gel filtration. The principal aim of ultrafiltration of whey is to concentrate the native or pre-denatured whey proteins in order to obtain a whey protein powder by evaporation under reduced pressure and spray drying (Siso, 1996; Abd El-Salam et al., 2009; Tsakali et al., 2010). WPC has been produced in different protein levels (35-80%) (Table 3.4). WPC-35 products are mainly used as replacers for skim milk, and WPC-60 for egg white in a number of bakery and confectionery applications, whereas WPC-80 products are well known for their specific properties in meat and fish products (de Wit, 2001).

The lactose and mineral content in whey can be further reduced using a subsequent diafiltration, yielding high value retentate of about 85% protein (Walstra *et al.*, 2006; Abd El-Salam *et al.*, 2009; Tsakali *et al.*, 2010) (Walstra et al. 2005, Abd El-Salam et al. 2009, Aswani 2010, Tsakali et al. 2010).

Table 3.4. Composition of WPC with different protein content

	WPC (%, dm)			
Composition (%)	**35**	**50**	**65**	**80**
Moisture	3.8	3.8	3.8	3.8
Crude protein (Nx6.38)	36.2	52.1	63.0	81.0
True protein	29.7	40.9	59.4	75.0
Lactose	46.5	30.9	21.1	3.5
Fat	2.1	3.7	5.6	7.2
Ash	7.8	6.4	3.9	3.1
Lactic acid	2.8	2.6	2.2	1.2

dm: dry matter; (Tetra-Pak, 1995)

WPC finds varieties of applications in various food formulations due to its excellent nutritional and functional properties. However, the end use of WPC depends on its protein content and the composition of other constituents (Jayaprakasha and Yoon, 2005).

WPC are commonly added to bakery, dairy and meat products, chocolate, candies,

frozen dessert, sauces, whipped toppings, custards, mayonnaise, puddings, confectionaries etc., in order for replacing fat, binding water (preventing syneresis), given body, and forming and stabilizing foam, gel and emulsification (de Wit, 2001; Foegeding and Luck, 2002; O'Connor and O'Brien, 2002; Walzem *et al.*, 2002; Abd El-Salam *et al.*, 2009).

In dairy products, WPC improves the slicing properties of cheeses and the rheological characteristics of yogurts by strengthening the gel structure, to prevent the separation of serum phase and increasing the water-holding capacity (González-Martínez *et al.*, 2002; Sodini *et al.*, 2006; Guggisberg *et al.*, 2007; Aziznia *et al.*, 2008). WPC has been used to increase solids and protein content in milk and cheese and to replace fat in low-fat dairy products (Tunick, 2008).

Foods with the low biological value of protein such as bakery products and candies are fortified with WPC to increase their nutritional value. Also, WPC is intentionally used in some bakery products where the Maillard reaction is desired for aroma and color formation. (Parris *et al.*, 1993; Beckett, 2002; de Wit, 2002; Lucey, 2002; O'Connor and O'Brien, 2002; Saunders, 2002; Jyotsna *et al.*, 2007).

In sport drinks and beverages, undenaturated WPC with good solubility at wide pH ranges and smooth taste improves the structure as well as the nutritional value (de Wit, 2002). WPC also maintains the structure and strength of continue phase in margarines (Early, 1998).

The defatted WPC with 80% of protein content are specifically designed to replace egg white in foods such as meringue, frappes, whipped toppings and sponge cake that contain gel or foam structure. The advantage for using defatted WPC instead of egg white is that WPC cannot be overwhipped, which is a problem by using egg white (Kinsella and Whitehead, 1989; de Wit, 2001).

WPC is often added to meat and fish products to improve texture, bind flavor, and, increase water- and fat-holding capacities because softness, juiciness and body are important quality parameters for the products (Kinsella and Whitehead, 1989; de Wit, 2001; Foegeding and Luck, 2002; Singh, 2002).

WPC improves the structure and palatability of confectionery products by enhancing the miscibility of formula ingredients because of their emulsifying properties, and contribute to lightness during whipping and the structure of the products during cooking (de Wit, 2001).

3.2.2.2. Whey protein isolates (WPI)

WPI contains a protein content of 88-95%, and β-lactoglobulin and α-lactalbumin are its major constituents (Bonnaillie and Tomasula, 2008). WPI is a white to light cream-colored powder with a bland taste and clean flavor. It is prepared from the whey skimmed by microfiltration, and demineralized by ionic exchange, electrodialysis or nanofiltration. Further purification is performed via diafiltration or combination of ion exchange chromatography and ultrafiltration. Finally, the purified retentate is concentrated and spray dried (de Wit, 2001; Foegeding and Luck, 2002; Kelly, 2002; Ha and Zemel, 2003; Tsakali et al., 2010). The WPI produced via ion exchange chromatography contains less casein and glycomocropeptides compared to that produced via microfiltration (Abd El-Salam et al., 2009).

WPI functions as water-binding, gelling, emulsifying and foaming agents in food products. WPI gels has been reported stronger and more elastic than WPC gels because of the higher β-LG and lower fat, lactose, and phospholipids contents (Bonnaillie and Tomasula, 2008).

WPI is extensively used in sports and nutritional supplements including beverages, sports drinks and nutrition bars due to its high protein purity, mineral content and solution clarity (Foegeding and Luck, 2002; Singh, 2002; Sun *et al.*, 2007; Tunick, 2008).

WPI has been found to provide better emulsion stabilities in the chilled and frozen foods compared to casein and Tween-20 via the prevention of droplet coalescence by forming relatively thick interfacial layers around the droplets (McClements, 2008).

In set-type yogurt, WPI yields better consistency and microstructure (Guggisberg *et al.*, 2007). It is used for the production of some cheese types (e.g. Ricotta) (Lucey, 2002). Addition of WPI to muscle protein improves moisture retention (Tunick, 2008). WPI can be used as edible films to prevent lipid oxidation and to extent shelf-life of nuts, and to impart a smooth and glossy appearance to dried food or confectionery products (Dangaran and Krochta, 2008). Denaturated WPI at alkali conditions results in stringy texturized meaty fibrous products, which could be used in meat applications (Onwulata, 2008).

3.2.2.3. Whey protein hydrolysates (WPH)

WPH contains no less than 90% protein, and is produced from heated WPI by enzymatic hydrolysis or acids followed by drying. Hydrolysis alters the functional properties of WPH, and the degree of hydrolysis is dependent on the purpose of its use.

The degree of hydrolysis for ranges from 4% to 20%. (Foegeding and Luck, 2002; Abd El-Salam *et al.*, 2009; Archer, 2016).

Whey protein hydrolysis yields peptide mixtures, which improve the foaming ability and the emulsion stability, and reduce the aggregation in gel during thermal processing. WPH is also used to reduce allergenicity and increase the intestinal absorption. Therefore, it commonly finds the applications in infant foods and medical foods used for the patients with allergic symptoms to milk protein. In general, the higher the degree of hydrolysis, the more heat stable and hypoallergenic the WPH. Lower levels of hydrolysis are associated with improved functionality in foaming, emulsification and gelation (Siso, 1996; Foegeding and Luck, 2002).

3.2.2.4. Whey protein fractions

A growing demand for specific proteins for use in infant and medical foods, and sport drinks has encouraged processors to fractionate whey proteins. Several methods based on differential solubility at different pH, temperature and ionic strength have been developed to separate the two major whey proteins, β-lactoglobulin and α-lactalbumin in relatively rich fractions (Foegeding and Luck, 2002; Ng-Kwai-Hang, 2002; Huppertz *et al.*, 2006; Abd El-Salam *et al.*, 2009).

β-Lactoglobulin is the major whey protein. It comprises 50% of total whey proteins, and is rich in cysteine, a sulphur-containing amino acid. It is very resistant to acids and enzymes in stomach due to its globular structure (Ng-Kwai-Hang, 2002; ADPI, 2016).(Huppertz *et al.*, 2006; Abd El-Salam *et al.*, 2009). β-Lactoglobulin is found in WPI higher than in WPC, providing better water-binding, emulsifying and gelling properties (Ng-Kwai-Hang, 2002; Yadav *et al.*, 2015; ADPI, 2016). Also, it may bind to hydrophobic molecules such as fatty acids and retinol because of its great foaming and gelation properties (Heino, 2009). In general, β-Lactoglobulin is added to the food formulations for fortification (Walzem *et al.*, 2002). It is moderately heat-stable, and this property has been exploited in fish products, formulated foods, acidic protein fortified beverages, and meat processing (Kinsella and Whitehead, 1989).

α-Lactalbumin is a compact globular protein, and accounts for 25% of total whey protein. It is rich in essential amino acids such as lysine, leucine, threonine, tryptophan and cysteine. It is commonly added to infant formulas to resemble the protein composition of breast milk, and to sport drinks to balance the amino acid composition (Walzem *et al.*,

2002; ADPI, 2016). α-Lactalbumin binds calcium, which may stabilize the molecule against irreversible thermal denaturation (Kinsella and Whitehead, 1989). However due to the limited solubility, α-Lactalbumin is not suitable for the foods required gel or foam structure (Archer, 2016). α-Lactalbumin has been reported to support the defense mechanism and reduce the risk of cancers (Walzem *et al.*, 2002).

3.2.3. Lactose and its derivatives

Lactose, known as the milk disaccharide, is composed of one glucose and one galactose molecule. Lactose is an important source of dietary energy and enhances the intestinal absorption of calcium from foods (de Wit, 2001).

Whey contains 4-5% lactose, and is commonly used for the lactose production. The deproteinized, defatted and demineralized whey is condensed to 60-65% of solids before crystallization, separation and purification. Lactose obtained from whey can be in the form of crystal powder, syrup or concentrate (Harper, 1992; Muir, 2002; Schaafsma, 2002; Kellam, 2016).

Lactose has been used in a wide range of food products. For soups, sauces, instant drinks, confectionary products, spice mixes and meat products, lactose is used to reduce sweetness, to enhance aroma and color, to improve dispersibility and free-flowing, and to increase storage life. Reduced sweetness, delayed crystallization, improved color and aroma, better mouthfeel, texture and chewiness, and improved shelf life are the major reasons for using lactose along with sucrose, glucose and fructose in confectionery products. In bakery products, lactose increases the dough resistance to mixing and fermentation, improves the dough machinability, boosts the browning reactions for enhanced crust color and flavor, and improves emulsifying properties of shortenings resulting in higher volume, uniform cell structure and desirable texture. Other applications of lactose are the coating of food particles and encapsulating liquid flavoring and coloring materials (Harper, 1992; de Wit, 2002; Schaafsma, 2002; Kelly, 2007).

Hydrolyzed lactose products by enzymes or acid/heat treatment have an opportunity to produce a range of products with specific functional properties. They overcome lactose intolerance, and increase sweetness and solubility, because lactose itself is rarely absorbed by humans, comparatively low in sweetness, hardly soluble in water, and tends to crystalize at high concentration levels with a resulting 'sandy' mouthfeel (Harper, 1992; Mahoney, 2002; Muir, 2002; Schaafsma, 2002; Walzem *et al.*, 2002; Kellam, 2016).

Hydrolyzed lactose has been used in (i) ice cream to replace sucrose, (ii) yogurt to stimulate development of the lactic acid bacteria, increase acetaldehyde production, improve hydrophilic properties and structure, and impart a smoother texture, (iii) confectionary products to increase chewiness, reduce lactose crystallization, and improve darker color and flavor, (iv) bakery products to increase volume and porosity, improve crumb compressibility and reduce fermentation time, and (v) beverages to avoid problems of protein precipitation. (Harper, 1992; Zadow, 1992).

Lactulose, lactitol, lactobionic acid, lactosyl urea and lactose-containing oligosaccharides are the main lactose derivatives, and obtained by enzymatic conversion, or hydrogenation or isomerization in the presence of metal catalysts. They have advantages over lactose as less or more sweetness, higher solubility, lower absorption, and higher stability at low pH's and high temperatures (Harper, 1992; Zadow, 1992; Walzem *et al.*, 2002).

3.3. Butter

Butter is a traditional food used worldwide and essential for human nutrition. Due to its high fat content, it is an important source of energy and contains many other nutritionally important components, such as saturated and unsaturated fat acids, phospholipids, cholesterol, minerals and fat-soluble vitamins which support overall body function, along with various health benefits (Göktürük *et al.*, 2002; Kwak *et al.*, 2013).

The main quality parameters that characterize butter are fat and water contents (Dvořák *et al.*, 2016). Butter is a water-in-oil emulsion with a minimum fat content of 80%, in which water content should not exceed 16% and non-fat milk solids generally constitute 2% (TGK, 2005). The composition and the rheological properties of butter vary greatly depending on the stage of lactation, diet, dietary supplementation, season of the year and diseases (Hawke and Taylor, 1983; Nickerson, 1995; Fox, 2000).

Butter products are broadly classified as sweet cream (unsalted/salted), cultured (unsalted/salted), or traditional sour cream butter. Lactic acid and flavor producing microorganisms (*Lactococcus lactis* subsp. *lactis* and *cremoris*, *Lactococcus lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*) yield cultured cream butter, in contrast to `sweet cream' butter which is made of uncultured cream. The flavor of sweet cream butter is mild and creamy, that of cultured butter is more intense (Frede, 2002).

For manufacturing commercial butter "continuous buttermaking" is typically used but the traditional small-scale on-the-farm method is the batch procedure, using rotational or upside-down churning. The churning process disrupts the membrane surrounding the fat

globules, and releases the free fat from the globules, ultimately resulting in a solid fat phase (butter), and an aqueous phase (buttermilk) (Frede, 2002).

Whey contains 0.3-0.7% of fat depending on the type and manufacturing processes of cheese. It adversely affects the functional properties of whey protein products e.g., water holding, foaming, emulsification, thickening and gelling. Therefore, it must be removed before the production of whey protein products. Recently, whey has been considered another source of butter manufacturing. Because of its low fat content, whey is centrifuged several times to obtained a cream with a fat content of 45-50%, which is heated and acidified to manufacture of butter (González-Martínez *et al.*, 2002; Abd El-Salam *et al.*, 2009).

4. MATERIALS AND METHODS

4.1. Materials

All chemicals were of analytical reagent grade, and obtained from the local distributer of Sigma (Germany). Deionized distillated water was used throughout. All working standard solutions were prepared immediately before use.

Whey butters were prepared from whey provided by four collection centers located in different cities, namely, Uşak, Adana, Burdur and Konya. The chemical compositions of whey samples are given in Table 4.1. In addition, two unsalted milk butters commercially available were used for the comparison purpose.

Table 4.1. Chemical composition of whey (W) samples

	Uşak (W-1)	**Adana (W-2)**	**Burdur (W-3)**	**Konya (W-4)**
pH	6.00 [a]	6.10 [a]	5.93 [a]	6.00 [a]
Titratable acidity (%, lactic acid)	0.28 [a]	0.29 [b]	0.32 [c]	0.28 [a]
Water (%, w/w)	93.53 [a]	93.39 [a]	93.86 [b]	93.70 [b]
Fat (%, w/w)	0.45 [b]	0.44 [b]	0.34 [a]	0.35 [a]
Non-fat dry matter (%, w/w)	6.20 [c]	6.17 [c]	5.80 [a]	5.95 [b]
Salt (%, w/w)	0.35 [b]	0.26 [a]	0.45 [c]	0.51 [c]

(Kasapçopur, 2016)

4.2. Whey Butter Production

Whey butters used in this study was obtained from the previous study (Kasapçopur, 2016). Briefly, whey sample was clarified at 5900 rpm (GEA, Denmark), batch-pasteurized (GEA, Denmark) at 80-85 °C for 15-20 s, and then passed through a fat separator (Haus, Turkey) multiple times until the fat content of cream was reached to 45-50%. The cream was batch-pasteurized at 90 °C for 15-20 s followed by cooling to 8 ^{o}C. The cream was allowed to crystallize for 3 h, and immediately churned into butter (GEA, Denmark). The

churning process took for 10 min. The butter grains were mixed continuously with cold water (6-8 °C) to drain out buttermilk. Finally, the butter was wrapped in aluminum foil, vacuum-packed in pouches and kept at 4 ^{o}C until further analysis (Figure 4.1). The chemical composition of butters is given in Table 4.2.

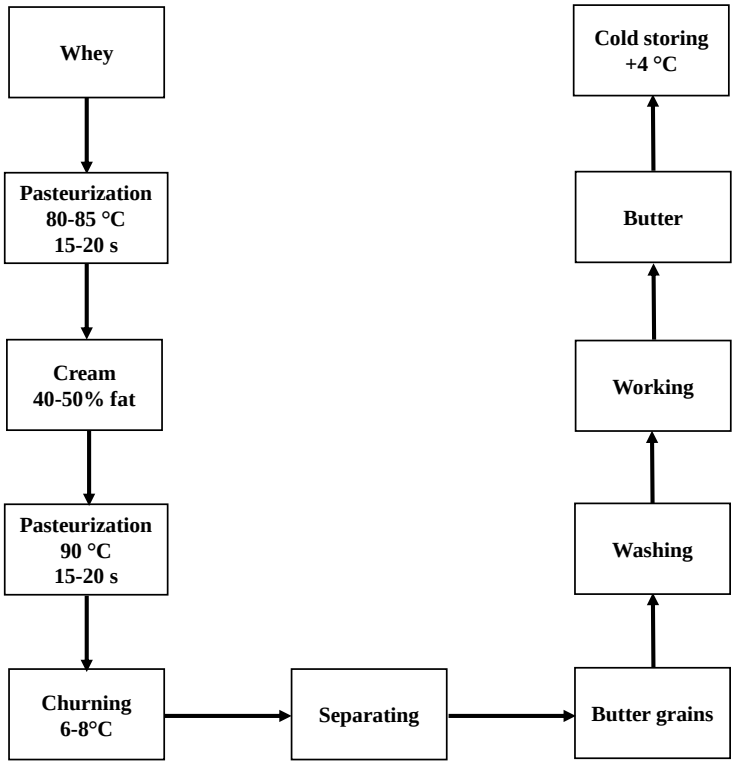

Figure 4.1. Whey butter production steps

Table 4.2. Chemical composition of butter samples

	Uşak (WB-1)	Adana (WB-2)	Burdur (WB-3)	Konya (WB-4)	MB-1	MB-2
Titratable acidity (% lactic acid)	0.41	0.47	0.53	0.55	0.40	0.41
Moisture (%, w/w)	20.01	18.83	20.61	19.87	15.25	15.20
Fat (%, w/w)	79.27	79.97	78.23	79.47	82.97	82.93
NFDS (%, w/w)	0.73	1.20	1.16	0.66	1.78	1.86
Salt (%, w/w)	0.09	0.06	0.11	0.13	0.07	0.07

(Kasapçopur, 2016); WB; whey butter; MB, milk butter; NFDS, non-fat dry solids

4.3. Protein Content

Total protein content of butter samples were determined by Velp Kjeldahl UDK139 (Italy) (Figure 4.2) according to the Method No 991.20 (AOAC, 2012). Result is expressed as the percentage of protein (w/w) in total nitrogen basis after multiplying the total nitrogen by a factor of 6.35.

Figure 4.2. Velp UDK139 semi-automatic Kjeldahl distillation unit

4.4. Total Carotenoid and Total Chlorophyll

Carotenoid and chlorophyll contents of the butter samples were determined spectrophotometrically (Minguez-Mosquera *et al.*, 1991). Briefly, 7.5±0.25 g butter was weighed, dissolved in cyclohexane, and taken to a final volume of 25 mL. The maximum absorption was determined for carotenoid at 470 nm and chlorophyll fractions at 670 nm, using a Lambda 45 spectrophotometer (Perkin Elmer, USA) (Figure 4.3). The coefficients of specific extinction for carotenoid and chlorophyll were taken as 2000 and 613, respectively. Their concentrations were calculated as follows:

$$\text{Total Carotenoid (mg/kg)} = \frac{A_{470} \times 10^6}{2000 \times 100 \times L} \quad (4.1)$$

$$\text{Total Chlorophyll (mg/kg)} = \frac{A_{670} \times 10^6}{613 \times 100 \times L} \quad (4.2)$$

Where A is the absorbance and L is the spectrophotometer cell thickness (1 cm). The data reported is based on butter weight.

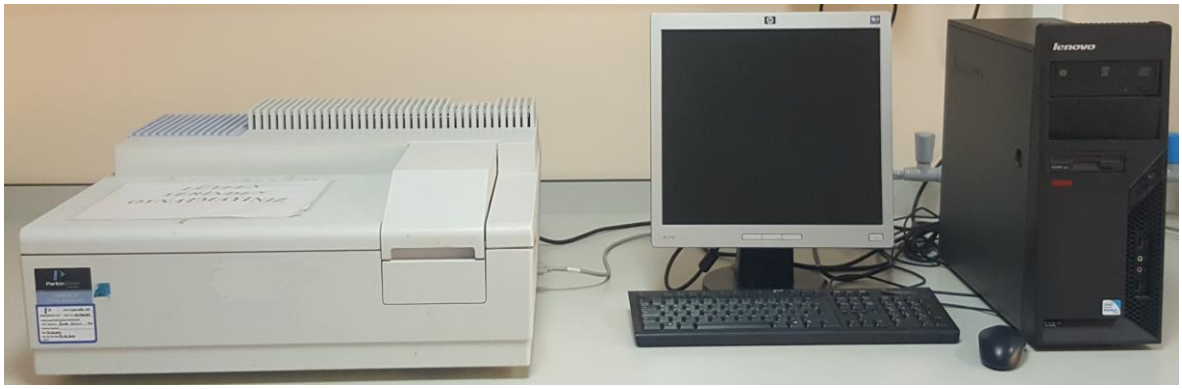

Figure 4.3. Perkin Elmer Lambda 45 spectrophotometer

4.5. Tocopherol Isomers

Tocopherols in the butter samples were determined according the standard method of International Organization for Standardization (ISO, 2012). The butter samples (5±0.25 g) were dissolved in 25 mL *n*-heptane and centrifuged at 4000 rpm for 5 min. The supernatant was transferred to a 100-mL volumetric flask. The procedure was repeated twice with 25 mL *n*-heptane. The combined supernatants were filtered with a 0.45 µm PVDF filter (Pall Life Science, ABD), and evaporated to dryness under a nitrogen stream. The butter samples were reconstituted with the mobile phase immediately prior to analysis by HPLC.

Detection and quantification were carried out with a Shimadzu Prominence HPLC equipped with CBM-20A Prominence System controller, SIL-20AC Prominence Autosampler, LC-20AT Prominence pump and RF-10AXL fluorescent detector that was set at 295 nm for excitation and 330 nm for emission (Kyoto, Japan) (Figure 4.4). Chromatographic separation of the tocopherol isomers was achieved at 30 °C, using a 250 mm×4.6 mm×5 µm Luna Silica column with a 10×4.6 mm guard column (Phenomenex, USA). The mobile phase was consisted of n-heptane/Tetrahydrofuran (95/5)

(v/v) at a flow rate of 1.2 mL/min, and the injection volume was 20 µL. Tocopherol isomers were identified by comparing the retention times with those of authentic individual tocopherols (Sigma Chemical Co., St. Louis, MO, USA) dissolved in mobile phase (Figure 4.5), and expressed as mg per kg butter.

Figure 4.4. Shimadzu Prominence HPLC with RF-10AXL fluorescent detector

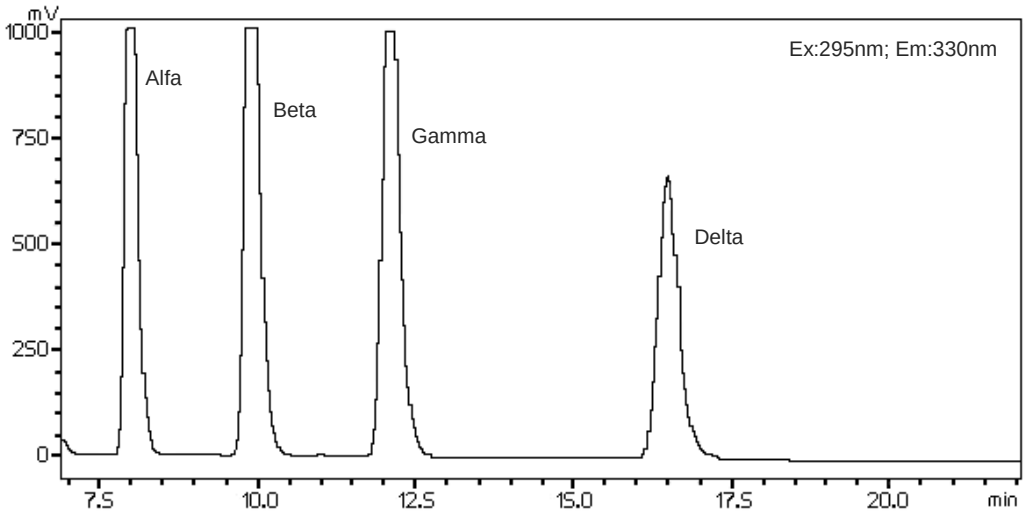

Figure 4.5. HPLC chromatogram of tocopherol isomers

4.6. Mineral Content

Total concentrations of five elements (Ca, K, Mg, Na, and P) in the butter samples

were determined according to the EPA standard method (EPA, 2014) by an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 2100DV, Perkin Elmer, USA) (Figure 4.6). Argon (99.999% of purity) was used as sample introduction and plasma gas. Butter samples (0.25±0.05 g) were microwave digested with 3 mL HNO3 (65%) and 2 mL H2O2 at up to 200 °C for 40 min in closed vessels. The microwave digestion was carried out in a Berghof MWS3+ (Germany) oven equipped with pressure and temperature control. Microwave experiments were done in closed DAP60 vessels. The microwave digestion program was applied as follows: Step 1: 160 °C, power 50%, 5 min; Step 2: 200 °C, power 75%, 5 min; Step 3: 100 °C, power 40%, 20 min. Each sample was digested in triplicate and analyses were carried out once on each digest. Prior to the ICP-OES analysis, the digested samples were diluted to 25 mL with ultra-pure laboratory Milli-Q water (Millipore, USA). The operating conditions for the measurements with ICP-OES are given in Table 4.3. Calibration curves were developed using standard solutions of 0.25-2 mg/kg obtained by dilution from a multi-element standard containing 1000 mL/L of Ca, K, Mg, Na, and P (Sigma Aldrich., USA) with HNO_3 (65%).

Figure 4.6. Perkin Elmer ICP-OES with microwave digestion system

4.7. Statistical Analyses

All analyses were replicated three times for each sample. All data was analyzed using one-way analysis of variance (ANOVA), and significant differences between means were measured with Duncan's multiple range tests at $p<0.05$ using the SPSS (ver.23) statistical program (IBM, ABD).

Table 4.3. Operating conditions for ICP-OES analysis

Operation Parameters		
RF power (W)		: 1450 W
Generator frequency (MHz)		: 40.68 MHz
Flash time		: 10 s
Reading time		: 30 s
Washing time		: 30 s
Plasma gas flow rate		: 17 L/min
Nebulizer gas flow rate		: 0.8 L/min
Auxiliary gas flow rate		: 0.2 L/min
Sample flow rate		: 1.5 mL/min
Spray chamber		: Rayton Scott
Nebulizer		: GemTip Crossflow
Injector		:2 mm (i.d.) Alumina
Wavelength (nm)		
	K	: 766.490
	Na	: 589.592
	Ca	: 317.933
	Mg	: 285.213
	P	: 213.617

5. RESULTS AND DISCUSSION

5.1. Total Protein Content

Protein is found in the aqueous phase of butter, and its volume fraction is very small. The protein content of butter samples ranged from 0.14 to 1.20% (Figure 5.1). The reported value of protein content for milk butter is between 0.24-1.10% (McPherson and Kitchen, 1981; Lindsay and Lovatt, 1994; Pham *et al.*, 1994; Willix *et al.*, 1998; Rossmann *et al.*, 2000; Morin *et al.*, 2006; Rombaut *et al.*, 2006; Nahid, 2007; Samet-Bali *et al.*, 2009), and the results for the milk butter samples in this study (1.20 and 1.13% for MB-1 and MB-2, respectively) are in agreement with the reported values.

According to one-way ANOVA, the difference in the protein content among the samples was found to be statistically significant ($p<0.05$). Duncan Multiple Range categorized the butter samples in three groups: (i) WB-1 and WB-2, (ii) WB-3 and WB-4, and (iii) MB-1 and MB-2. The whey butter samples had significantly lower protein content than the milk butter samples ($p<0.05$).

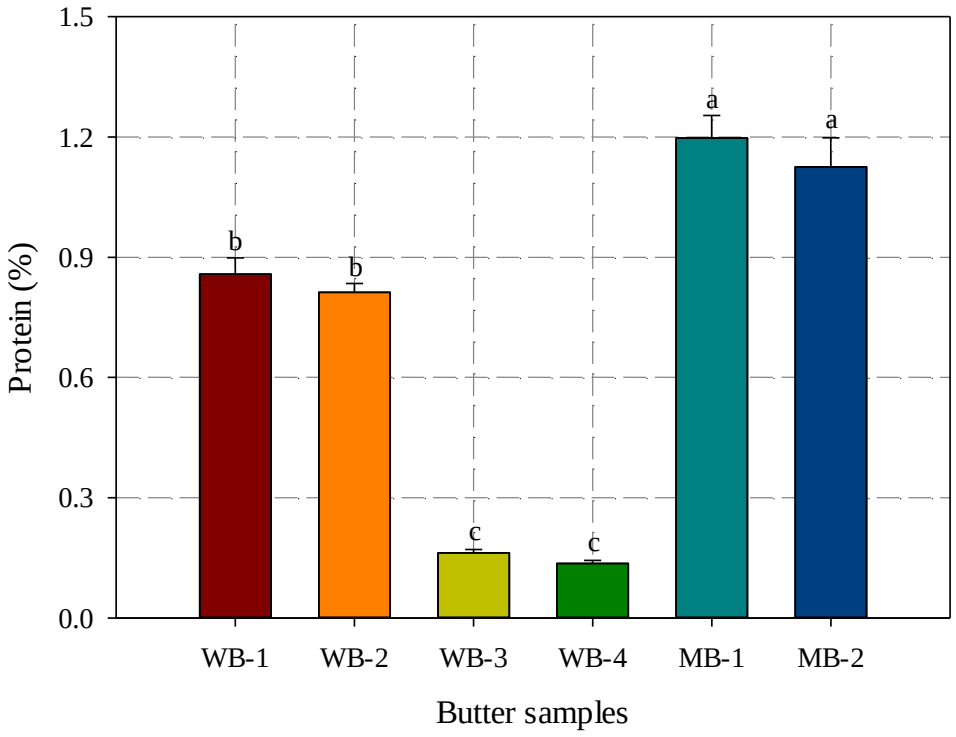

Figure 5.1. Protein contents of butter samples
(Different letters on bars indicate significant difference among butter samples at $p<0.05$)

Whey butters produced from the whey collected from Uşak (WB-1, 0.88%) and Adana (WB-2, 0.81%) had significantly higher protein content than those collected from Burdur (WB-3, 0.16%) and Konya (WB-4, 0.14%) ($p<0.05$).

In general, the protein content is directly related to the moisture content of butter, i.e., the higher the moisture content the higher the protein content. Although the milk butter samples used in this study (Table 4.2) (Kasapçopur, 2016) and the literatures (McPherson and Kitchen, 1981; Lindsay and Lovatt, 1994; Pham *et al.*, 1994; Willix *et al.*, 1998; Morin *et al.*, 2006; Rombaut *et al.*, 2006; Nahid, 2007) had similar moisture content (15-16%), they were different in their protein content. Nahid (2017) found no relation between the moisture and protein contents. Similarly, the whey butter samples in this study had high in the moisture content (18.83-20.61 %); however, low in the protein content (0.14-0.86%) compared to the milk butter samples. In addition, they showed variations within themselves depending on the collection center. Therefore, milk and whey compositions (nonfat solid content), and cheese and butter processing techniques probably affect the final protein concentration in butter. It was reported the changes in protein concentration of butter, as aff ected by the number of washing cycles (Britten *et al.*, 2008).

5.2. Total Carotenoid Content

The yellow coloration is generally considered an important quality attribute for milk products especially for butter, which is related to the carotenoid other similar pigment concentration in the butter (Nozière *et al.*, 2006; Agabriel *et al.*, 2007; Beliveau, 2012). Carotenoids are a family of fat-soluble pigments ranging from light yellow through orange to deep red. They are biosynthesized by all photosynthetic bacteria, cyanobacteria, algae, higher plants and by some non-photosynthetic bacteria, fungi, and yeasts. They are partially transferred into lipidic fraction of animal products, and contribute the nutritional and sensory characteristics of dairy products, particularly of milk, butter and some cheeses (Nozière et al., 2006; Beliveau, 2012). Among the carotenoids, β-carotene is an effective quencher of singlet oxygen and free-radical scavenger, and has been found to protect riboflavin against light-induced degradation and the lipids against photooxidation (Hansen and Skibsted, 2000; Chow, 2013).

Total carotenoid content of the butter samples is illustrated in Figure 5.2. The butter samples analyzed in this study contained 1.07-3.88 mg/kg of total carotenoid. According to one-way ANOVA, the difference in the carotenoid content among the samples was found

to

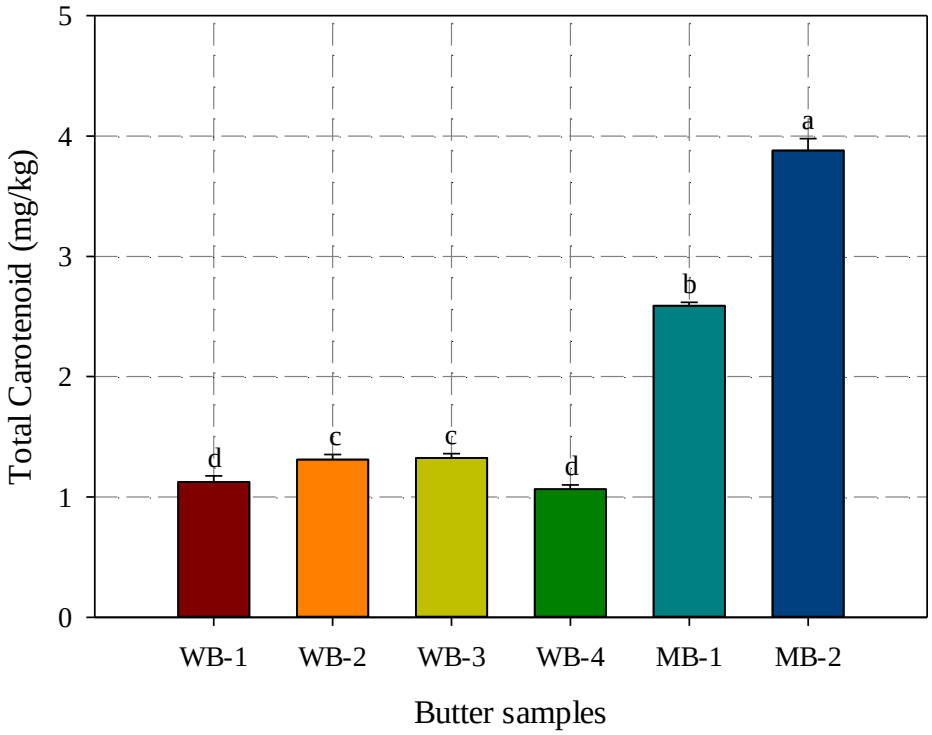

Figure 5.2. Carotenoid contents of butter samples
(Different letters on bars indicate significant difference among butter samples at $p<0.05$)

be statistically significant ($p<0.05$). MB-2 (3.88 mg/kg) contained more carotenoid than MB-1 (2.59 mg/kg) ($p<0.05$). This difference in milk butters is probably related to differences in the milk composition and butter manufacturing process.

The whey butter samples were relatively low in carotenoid content compared to the milk butter samples ($p<0.05$). Kasapçopur (2016) reported that butters made from the milk were more yellow color than those made from whey according to his Hunter Lab analyses. The whey butters produced from the whey collected from Adana (WB-2, 1.31 mg/kg) and Burdur (WB-3, 1.33 mg/kg) had significantly higher carotenoid content than those collected from Uşak (WB-1, 1.13 mg/kg) and Konya (WB-4, 1.07 mg/kg) ($p<0.05$). However, the similarity of the carotenoid content was found between WB-1 and WB-4, and between WB-2 and WB-3 ($p>0.05$).

The carotenoid concentration in butter reported in the literature vary greatly among studies, ranging from 0.38 to 8.20 mg/kg (Berl and Peterson, 1945; Herzer and Gieger, 1946; Shukla *et al.*, 1994; Göktürük *et al.*, 2002; Hulshof *et al.*, 2006; Belitz *et al.*, 2009a; Beliveau, 2012; Gaucheron, 2013; O'Callaghan *et al.*, 2016; Kahyaoğlu and Çakmakçı,

2018). Several factors have been identified to account for the variability in the carotenoid content such as animal species, breeding, lactation, health status, heritability, season, nature of forage, production factors, storage conditions, milk yield, fat content and microorganisms (Göktürük *et al.*, 2002; Nozière *et al.*, 2006; Beliveau, 2012; O'Callaghan *et al.*, 2016; Kahyaoğlu and Çakmakçı, 2018). Data in this study for the milk butter samples are within the range found in the literatures.

Carotenoids are sensitive to a number of different physical and chemical factors including air, oxidizing agents, ultraviolet light, pH and temperature. Consequently, the processing of milk to produce butter or cheese, which involves both heating and acidification, as well as the packaging and storage environment may result in degradation of carotenoids. In addition, some may be lost in the cheesemaking process. Although carotenoids are fat soluble, not all fat in milk is retained in the cheese produced, and a small proportion of carotenoids have been shown to be associated with whey proteins. 5-40% of carotenoids have been reported to be lost to the whey during the process (Lucas *et al.*, 2006; Agabriel *et al.*, 2007; Beliveau, 2012). This explains the low content of carotenoids in whey butters.

5.3. Total Chlorophyll Content

Recent studies indicated that protoporphyrin, hematoporphyrin, chlorophyll-a and chlorophyll-b are found naturally in milk and dairy products (Wold and Lundby, 2007). The presence of chlorophyll in dairy products can be explained by the diet of milking animals. The animals fed with green pastures contain more chlorophyll compounds in their milk. Besides, type of dairy products, storage and processing methods are effective on the variation in the chlorophyll content (Wold *et al.*, 2005; Wold and Lundby, 2007; Esmaeilifard *et al.*, 2016).

Although the concentrations of these compounds are very low in dairy products, they are sensitive to light and give significant contribution to photo-oxidation. The light-induced degradation of these compounds in dairy products, e.g. cheese, butter and dairy spreads, is highly correlated with oxidized odor and off-flavor (Hansen and Skibsted, 2000; Wold *et al.*, 2005; Andersen *et al.*, 2008).

Total chlorophylls were detected in the milk and whey butter samples by UV/Vis spectrophotometer at 670 nm and the result is presented in Figure 5.3. The total

chlorophyll content was 10.60 µg/kg for MB-1 and 2.45 µg/kg for MB-2, and ranged from 29.36 to

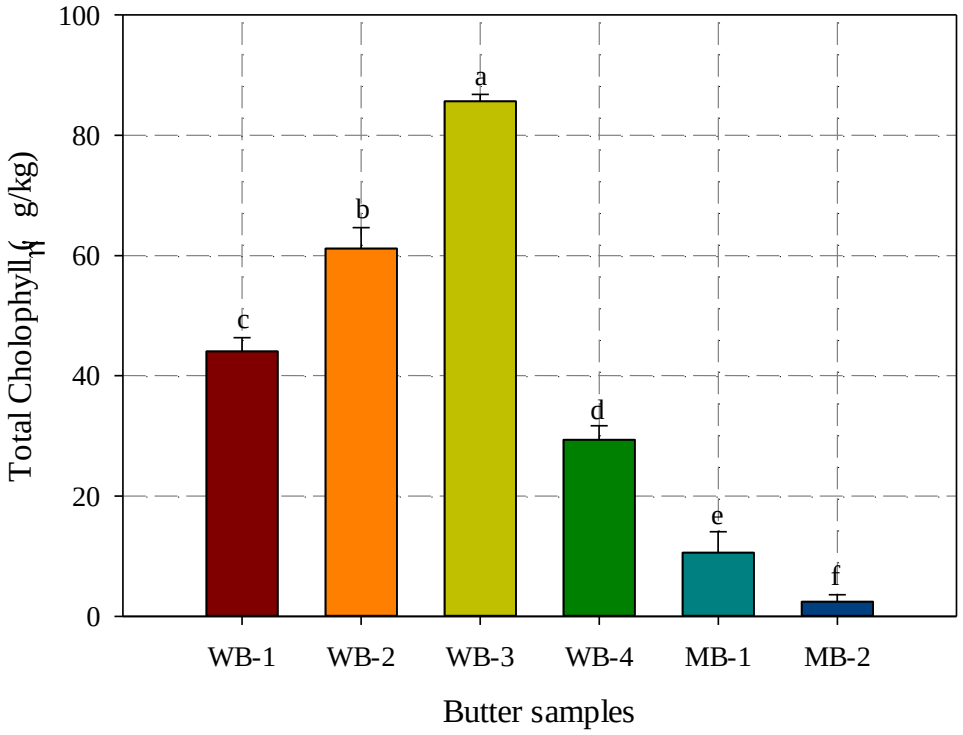

Figure 5.3. Chlorophyll contents of butter samples
(Different letters on bars indicate significant difference among butter samples at $p<0.05$)

85.64 µg/kg for the whey butters. From the statistical analyses, it was observed that all samples were significantly different from each other ($p<0.05$). In general, whey butters showed marked differences than milk butters ($p<0.05$). The highest chlorophyll content was found in the WB-3 obtained from the Burdur collection center while the lowest in the WB-4 obtained from the Konya collection center. WB-1 from the Uşak and WB-2 from the Adana collection centers had total chlorophyll contents of 44.05 and 61.17 µg/kg, respectively.

As a result of the literature search made in line with the possibilities, no reported value was found on the chlorophyll content of whey butter. For milk, however, only one study reported the chlorophyll content in butters as 0.12-0.17 mg/kg (Esmaeilifard et al., 2016). In comparison with this result, the milk and whey butters in this study had much more lower chlorophyll content. As indicated above, several factors, e.g. from animal spices through processing conditions can affect the chlorophyll in butters.

5.4. Tocopherol Isomers

Tocopherols are widely distributed in a variety of plants. Tocopherols consist of four tocopherols and four tocotrienols (α-, β-, γ- and δ-) isomers, and possess antioxidant activity. Tocopherols, as well as tocotrienols, can react with peroxy radicals and, therefore, are very effective to prevent autoxidation (Frankel, 1998; Chow, 2013; Desouky, 2014).

The contents of individual tocopherol isomers are presented in Table 5.1. In all tested butter samples, α-T was the predominant isomer, and constituted 93-95% of all tocopherols present. Similar results were found in the studies of Derewiaka *et al.* (2011), Gaucheron (2011), Chow (2013) and Górnas *et al.* (2014) who reported 90-95% of total tocopherol was in the form of α-T isomer. The study showed that the milk butter samples contained around 3% more α-T than the whey butter samples.

The proportion of the β-T in total tocopherol content was 1-2%. The concentration of the γ-T in the butter samples was 4-6% of all tocopherol isomers as reported by Stolyhwo and Rutkowska (2007). No δ-T was found in all tested butter samples. Schwartz *et al.* (2008) and Chow (2013) also reported the absence of δ-T in butter. Neupaney *et al.* (2003) noted that the β-T and δ-T isomers were not significantly detected in milk butter. However, Górnas *et al.* (2014) studied 12 butter samples by HPLC, and determined the presence of small quantities of δ-T (0.1-1.8 mg/kg) while they did not detect β-T. They reported the concentration of γ-T as 1.0-3.1 mg/kg. Due to the fact that butter is a natural product, its tocopherol composition might vary in animal type and breed, feeding regimes, seasons and processing conditions (Kanno *et al.*, 1968; Bauernfeind and Desai, 1977; Göktürük *et al.*, 2002; Górnaś *et al.*, 2014).

Table 5.1. Tocopherol contents of butter samples

	Tocopherol content (mg/kg)			
Samples	**α-T**	**β-T**	**γ-T**	**Total-T**
WB-1	25.56 ^{b}	0.40 ^{b}	1.06 ^{c}	27.02 ^{b}
WB-2	25.77 ^{b}	0.41 ^{b}	1.07 ^{c}	27.25 ^{b}
WB-3	25.53 ^{b}	0.40 ^{b}	1.41 ^{b}	27.33 ^{b}
WB-4	25.50 ^{b}	0.42 ^{b}	1.39 ^{b}	27.31 ^{b}
MB-1	26.37 ^{a}	0.41 ^{b}	1.73 ^{a}	28.50 ^{a}
MB-2	26.35 ^{a}	0.50 ^{a}	1.44 ^{b}	28.28 ^{a}

Means followed by different letters within same columns indicate significant differences among butter samples at $p<0.05$)

All butter samples from milk and whey contained 27.02-28.50 mg/kg of total tocopherol. Tocopherol content was higher in the milk butters than the whey butters ($p<0.05$). Duncan's multiple comparison test indicated, however, there was no significant difference between the milk butter samples and among the whey butter samples ($p>0.05$).

The reported concentration for total tocopherol ranged from 10 to 50 mg/kg with the average value of 25 mg/kg (Atuma, 1975; Hewavitharana *et al.*, 1996; Zamarreño *et al.*, 1999; Göktürük *et al.*, 2002; Neupaney *et al.*, 2003; Stolyhwo and Rutkowska, 2007; Schwartz *et al.*, 2008; Belitz *et al.*, 2009b; Derewiaka *et al.*, 2011; Chow, 2013; Gaucheron, 2013; Górnaś *et al.*, 2014). The results obtained in this study are well correlated to those given in the literatures for butters produced from milk.

5.5. Minerals

Minerals are involved in a large number of physiological functions and provide several health benefits when supplied in adequate amounts. Milk and dairy products serve as substantial sources of a number of minerals. In milk and dairy products, there are various minerals compromising small fraction of milk (about 8-9 g/L) that include calcium, magnesium, sodium, potassium, phosphorus, zinc, selenium, citrate and chloride. The milk minerals are considered crucially important not only for nutritional value, but also for functionality (gelation, formation and stability of the casein micelles, acid-base buffering, emulsification, foaming and cheese texture) in various dairy foods (Lucey and Fox, 1993; Johansen *et al.*, 2002; Lucey and Horne, 2009; Bonjour and Lecerf, 2011; Rodrigues, 2013).

The presence and concentration of minerals in milk is influenced by a number of factors, including animal spices, breeding, genetics, lactation, animal health, nutrition season and processing (Lucey and Horne, 2009; Zamberlin *et al.*, 2012). The partition of salts between the colloidal (micellar) and serum (soluble) phases is shown in Table 5.2.

The distribution between colloidal and serum phases depends on pH, temperature, and concentration. In the serum phase, milk minerals may be present as ion pairs. The Ca and Mg in milk are present at low concentrations as free ions, some as complexes with citrate and phosphate as well as significant amounts associated with casein micelles. Both Mg and citrate are present in the colloidal phase, which is remarkable since their concentrations (or activities) are not in excess of solubility. The Ca and phosphate contents

vary in proportion to the casein content of milk since much of the Ca and phosphate are associated with the casein micelles (Gaucheron, 2005; Lucey and Horne, 2009; Zamberlin *et al.*, 2012).

Minerals in serum phases (whey) have a major effect on the functionality of whey products, i.e., mechanical and sensory properties of whey protein (Lucey and Fox, 1993; Havea *et al.*, 2001; Lucey and Horne, 2009; Rodrigues, 2013).

Table 5.2. Distribution of salts between the colloidal and serum phases in milk

	Colloidal (micellar) (%)	**Serum (soluble) (%)**
Calcium	69	31
Phosphorus	53	47
Magnesium	47	53
Potassium	6	94
Sodium	5	95

(Fox and Mcsweeney, 1998; Lucey and Horne, 2009)

5.5.1. Calcium

Calcium is one of the major minerals present in milk and dairy products. In cow milk, Ca concentration is 1040-1340 mg/L (Gaucheron, 2005; Cashman, 2006; García *et al.*, 2006; Lante *et al.*, 2006; Reykdal *et al.*, 2011; Zamberlin *et al.*, 2012), and distributed between the micellar (about 800 mg/L) and aqueous phase (about 400 mg/L) (Gaucheron, 2011). About 40% of Ca exists in true solution, with 60-70% being associated with casein in a colloidal suspension (Huppertz *et al.*, 2006; Lucey and Horne, 2009; Gaucheron, 2011; Zamberlin *et al.*, 2012). The major forms of Ca in milk are calcium phosphate, calcium phosphocaseinate and calcium citrate (Hazell, 1985).

Ca concentration of the butter samples is shown in Figure 5.4. The butter samples analyzed in this study contained 73.12-174.30 mg/kg of Ca. No significant difference was found between the milk butter samples ($p>0.05$). The Ca concentration in milk butter reported in the literature was ranged from 87 to 221 mg/kg (Kohiyama *et al.*, 1993; Ieggli *et al.*, 2011; Zamberlin *et al.*, 2012). In general, the Ca concentrations obtained in this study for the milk butter samples are within the normal values described in the literature.

According to one-way ANOVA, the difference in the Ca concentration among the milk and

whey butter samples was found to be statistically significant (p<0.05). The milk butter samples (169.55 and 174,30 mg/kg for MB-1 and MB-2, respectively) had almost a 2-fold higher Ca concentration than the whey butter samples. When comparing the Ca concentration in whey butter samples obtained from different whey collection centers, there was a notable difference. The whey butter produced from the whey collected from Konya (WB-4, 94.72 mg/kg) had the highest Ca concentration followed by Adana (WB-2, 92.65 mg/kg), Burdur (WB-3, 83.40 mg/kg) and Uşak (WB-73.12 mg/kg). However, from the statistical point of view, WB-2 and WB-4 were found to be different than WB-1 (p<0.05).

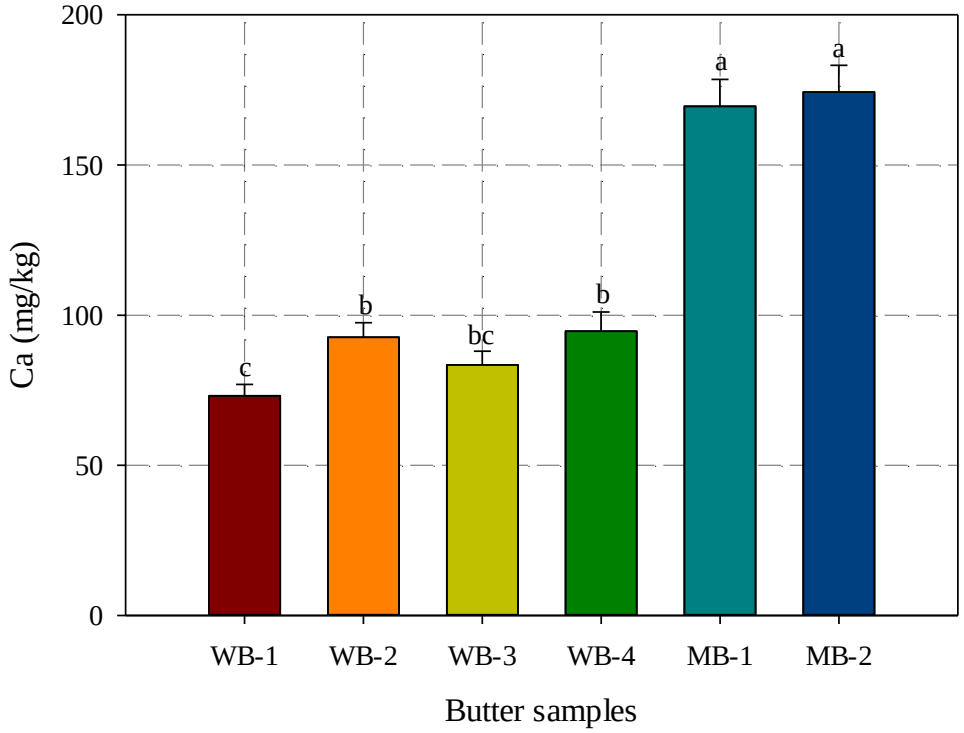

Figure 5.4. Calcium contents of butter samples
(Different letters on bars indicate significant difference among butter samples at p<0.05)

Discrepancies in the Ca concentrations in milk and whey butter samples can be explained by differences in the technological processes (manufacture of butter and manufacture of cheese). Casein coagulates when Ca is available. This reaction is the key to obtain rennet-induced and acid-induced gels for cheese and yogurt production.

Acidification by either rennet or acids is the main modification in the Ca concentration, in which Ca is solubilized and transferred to aqueous phase (whey) (Lucey

and Fox, 1993; Gaucheron, 2011, 2013). It has been reported that the Ca concentration is 30-40% lower in whey than in milk (250-600 mg/L) (Fox and Mcsweeney, 1998; Lucey and Horne, 2009; Gaucheron, 2013). Also, Ca concentration lower in sweet whey (acidification via rennet) than in acid whey (direct acidification), suggesting some binding by whey proteins, especially by α-lactalbumin and osteopontin and casein at a lesser extent. Other factors such as seasonal variations, feeding, lactation and health status could also influence the amount of total Ca in the butter samples (Havea *et al.*, 2001; Gaucheron, 2013).

5.5.2. Phosphorus

Phosphorus is an essential nutrient for humans and serves a number of important biological functions (Flynn, 1992). P is also an important element present in milk and dairy products. Milk contains 840-1500 mg/L P that is present as organic and inorganic forms of P in serum and micellar phases (Hazell, 1985; Lucey and Fox, 1993; de la Fuente *et al.*, 1996; Gaucheron, 2005; Cashman, 2006; Huppertz *et al.*, 2006; Lante *et al.*, 2006; Barłowska *et al.*, 2011; Zamberlin *et al.*, 2012).

In milk, organic P is mainly associated with casein molecules in the micellar phase. The other forms of organic P are phospholipids, nucleosides, nucleotides and sugar phosphate) which are in the aqueous phase. Inorganic P is distributed between aqueous and micellar phases, and contributes to the mineral equilibrium of milk. At pH 6.7 of milk, inorganic P is located 50% in the aqueous phase and 50% in the micellar phase, forming nanoclusters of Ca phosphate. During the transformation of milk into dairy products, inorganic P is transferred to the aqueous phase, especially during acidification (Lucey and Fox, 1993; Gaucheron, 2005, 2013). The inorganic phosphates may also be combined with Ca, Mg or K (Hazell, 1985).

The P concentration of the butter samples varied from 56.03 to 142.70 mg/kg (Figure 5.5), and the milk butter samples is markedly higher P content. ANOVA relieved significant differences in the P concentration of the butter samples ($p<0.05$). The MB-2 had the highest while WB-2 had the lowest P content.

The difference between MB-1 (129.45 mg/kg) and MB-2 (142.70 mg/kg) was significant ($p<0.05$). The P concentration in the milk butter samples are within the limits reported by Kohiyama *et al*. (1993) (118-359 mg/kg), but lower than those reported by Belitz *et al*. (2009) (210 mg/kg), Reykdal *et al*. (2011) (240 mg/kg) and Zamberlin *et al*.

(2012) (240 mg/kg). This suggests that the source of milk, pH, season, animal health, feeding and butter production techniques may influence the P concentration in the milk butter samples.

The milk butter samples had more than 2-fold higher P concentration than the whey butter samples. The overall result of the whey butter samples showed relatively constant but some variations were observed. Among the whey butter samples, WB-3 from the Burdur whey collection center (72.20 mg/kg) contained the highest P content compared to WB-1 from Uşak (57.39 mg/kg), WB-2 from Adana (56.03 mg/kg) and WB-4 from Konya (66.74 mg/kg). However, the differences between WB-3 and WB-4 and among WB-1, WB-2 and WB-4 were insignificant (p>0.05).

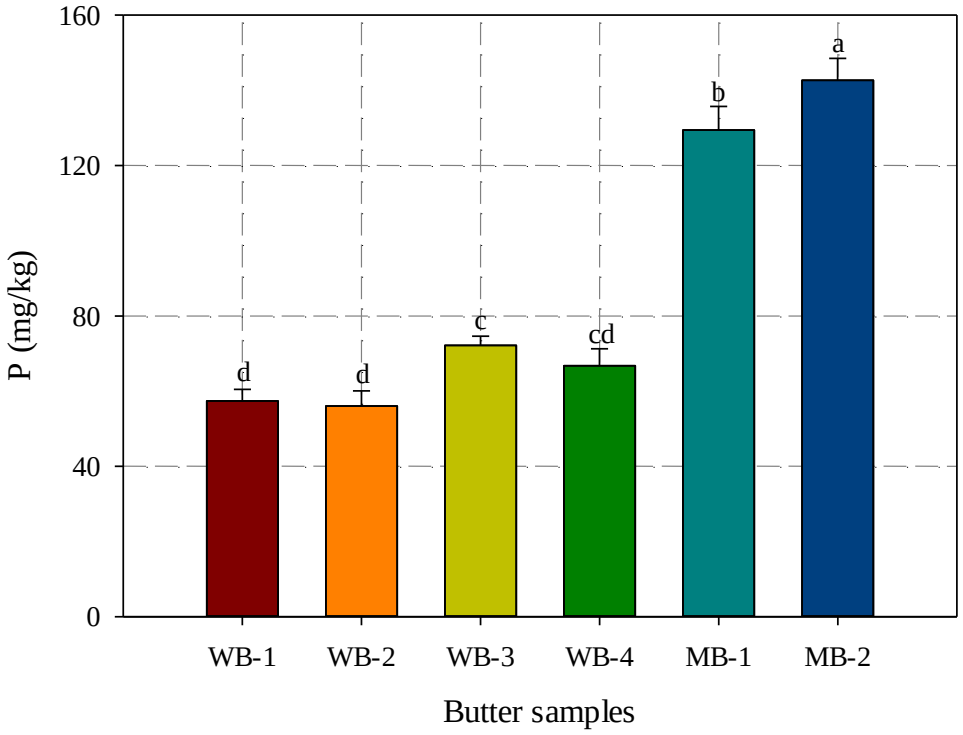

Figure 5.5. Phosphorus contents of butter samples
(Different letters on bars indicate significant difference among butter samples at p<0.05)

It has been reported that the P concentration in whey is dependent on the type (soft, semi-hard and hard) and manufacture of cheese (rennet or acid coagulation). During the cheese processing, significant amount of P (45-90%) is removed in the whey during draining, which is dependent on the pH level (Lucey and Fox, 1993; Fox and Mcsweeney, 1998; Lucey and Horne, 2009; Gaucheron, 2013). The P concentration in the literature is given as 400-580 mg/L). This explains the lower P concentration in the whey butters, and

indicates that the whey used in this study for the butter production is mostly sweet whey gained from the rennet-type coagulation to produce hard cheeses. It should also be taken into account that certain amount of P is bond by casein micelles and whey proteins, e.g., β-lactoglobulin.

5.5.3. Magnesium

Magnesium has an essential role in a wide variety of physiological processes, including protein and nucleic acid metabolism, neuromuscular transmission, and muscle contraction, and it acts as a cofactor for many enzymes (Flynn, 1992; Gaucheron, 2013). Mg is not abundant in milk and dairy products, and its concentration in milk is 70-220 mg/L depending on the source of milk (Lucey and Fox, 1993; de la Fuente *et al.*, 1996; Cashman, 2006; García *et al.*, 2006; Huppertz *et al.*, 2006; Lante *et al.*, 2006; Szefer and Grembecka, 2007; Barłowska *et al.*, 2011; Gaucheron, 2011; Reykdal *et al.*, 2011; Zamberlin *et al.*, 2012; Gaucheron, 2013; Bilandžić *et al.*, 2015). Mg is associated with inorganic phosphate and citrate in the aqueous phase (70 mg/L) and in the micellar phase with casein (50 mg/L).

The Mg concentration of the butter samples was found to be in the range of 12.83-22.09 mg/kg. Figure 5.6 illustrates that the milk butter samples (MB-1, 22.09 and MB-2, 21.09) are high in the Mg concentration compared to the whey butter samples. In the literatures, the Mg content of milk butter was ranged from 17 to 36 mg/kg (Szefer and Grembecka, 2007; Reykdal *et al.*, 2011; Zamberlin *et al.*, 2012; Gaucheron, 2013; Bilandžić *et al.*, 2015). The results obtained in this study for the milk butter samples are comparable with the reported values.

ANOVA showed that WB-4 made from the whey obtained from the Konya collection center with 20.12 mg/kg of Mg concentration did not differ significantly from the milk butter samples ($p>0.05$). WB-1 from Uşak (12.83 mg/kg) and WB-2 from Adana (13.25 mg/kg) had a similar Mg concentration ($p>0.05$), which was lower than the Mg concentration of MB-3 from Burdur (17.49 mg/kg) ($p<0.05$).

In dairy products, the concentrations of Mg vary depending on the manufacturing process. The distribution of Mg between micellar and aqueous phases is sensitive to pH, thus, during milk acidification, micellar Mg is solubilized in the aqueous phase of acidified milk (Gaucheron, 2013). Around 50% of Mg retains in whey after cheese manufacturing (de la Fuente *et al.*, 1996; Fox and Mcsweeney, 1998; Johansen *et al.*, 2002; García *et al.*,

2006; Szefer and Grembecka, 2007; Lucey and Horne, 2009). The whey butters analyzed in this study contained more moisture content (18.8-20.6%), due to more successive working (washing) procedure with circulating chilled water than the milk butters (15.2%). This explains reasonably lower Mg concentration found in the whey butters.

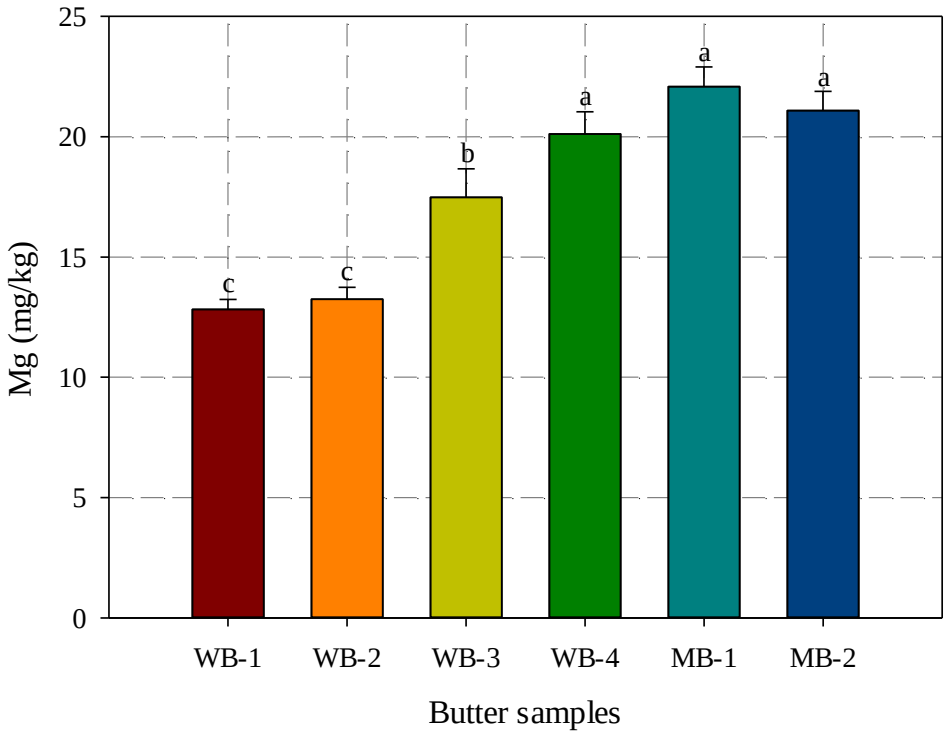

Figure 5.6. Magnesium contents of butter samples
(Different letters on bars indicate significant difference among butter samples at $p<0.05$)

5.5.4. Potassium

Potassium is the principal intracellular cation, contributing to the transmission of nerve impulses, controlling skeletal muscle contraction, activation of enzymes and regulation of blood pressure (Flynn, 1992; Gaucheron, 2013).

Figure 5.7 shows K level in the milk and whey butter samples. The results showed considerable differences in the butter samples. Significant higher K concentration was found in the milk butters than the whey butters ($p<0.05$). The milk butter samples (MB-1, 187.35 mg/kg and MB-2, 194.10 mg/kg) had a similar K concentration ($p>0.05$). The reported value for the K concentration in milk butter ranges between 104-330 mg/kg (Kohiyama *et al.*, 1993; Szefer and Grembecka, 2007; Abd El-Salam *et al.*, 2009; Belitz *et al.*, 2009a; Ieggli *et al.*, 2011; Reykdal *et al.*, 2011; Zamberlin *et al.*, 2012; Gaucheron,

2013). The obtained K results for the milk butters in this study are in good agreement with the reported values.

Among the whey butter samples, WB-3 from Burdur (135.95 mg/kg) and WB-4 from Konya (140.10 mg/kg) gave a significantly higher K level compared to WB-1 from Uşak

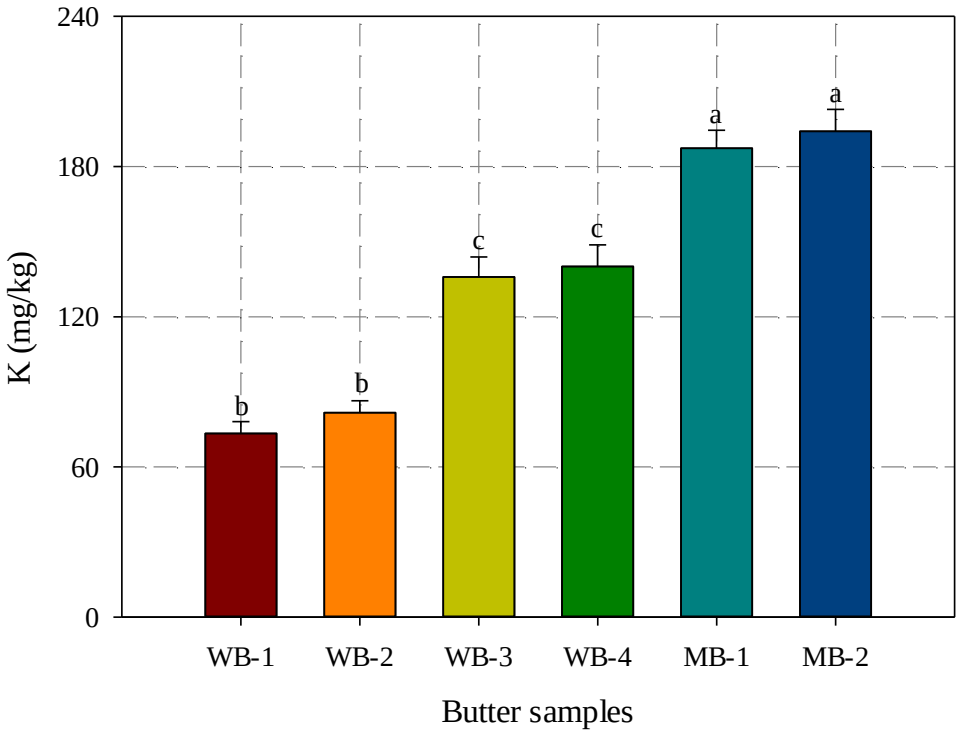

Figure 5.7. Potassium contents of butter samples
(Different letters on bars indicate significant difference among butter samples at $p<0.05$)

(73.36 mg/kg) and WB-2 from Adana (81.66 mg/kg) ($p<0.05$). However, there were no significant difference observed between WB-1 and WB-2, and between the WB-3 and WB-4 ($p>0.05$). The variation among the whey butter samples may be due to the milk composition, cheese processing and type of cheese produced. The concentration of K in milk is around 1500 mg/kg, and mainly located in the aqueous phase as free or weakly associated with ions of opposite charge. (90-95%) (de la Fuente *et al.*, 1996; Gaucheron, 2005; Cashman, 2006; García *et al.*, 2006; Huppertz *et al.*, 2006; Lante *et al.*, 2006; Lucey and Horne, 2009; Barłowska *et al.*, 2011; Reykdal *et al.*, 2011; Zamberlin *et al.*, 2012; Bilandžić *et al.*, 2015). Thus, dairy products such as cheese and butter are low in K, in which whey and buttermilk are drained away from the products.

5.5.5. Sodium

Sodium is the principal cation of extracellular fluid and is the primary regulator of extracellular fluid volume. It is important in the regulation of osmolarity, acid-base balance, and the membrane potential of cells, as well as in active transport across cell membranes. It has been estimated that milk and dairy products provide 20% of total sodium in European countries (Flynn, 1992).

The concentration levels of Na measured in the milk and butter samples given in Figure 5.8. The study showed a variation in the Na concentration of the butter samples. ANOVA classified the butter samples in three groups, which differed significantly from each other (p<0.05). The highest value was obtained in WB-3 (157.60 mg/kg) and WB-4 (165.85 mg/kg), while the lowest in the WB-1 (66.63 mg/kg) and WB-2 (63.95 mg/kg). The differences were insignificant between WB-3 and WB-4, between WB-1 and WB-2, and between MB-1 (95.66 mg/kg) and MB-2 (89.83 mg/kg) (p>0.05). The Na concentration in both milk butters is in line with the previous studies where Na concentration in milk butter has been reported between 46-110 mg/kg (Kohiyama *et al.*, 1993; Belitz *et al.*, 2009a; Ieggli *et al.*, 2011; Zamberlin *et al.*, 2012; Gaucheron, 2013; Bilandžić *et al.*, 2015).

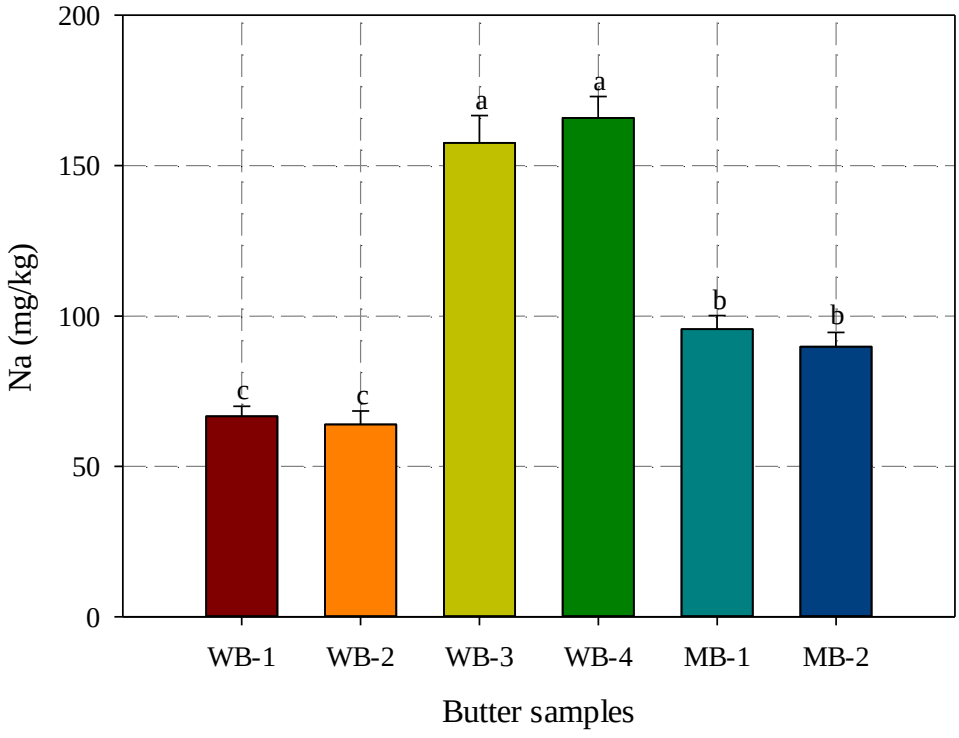

Figure 5.8. Sodium contents of butter samples
(Different letters on bars indicate significant difference among butter samples at p<0.05)

The variation in the Na concentration among the whey butter samples is probably related to cheese manufacturing. Milk contains 320-600 mg/kg of Na, which is mainly located in the aqueous phase (95%) of milk and dairy products (de la Fuente *et al.*, 1996; Gaucheron, 2005; Cashman, 2006; García *et al.*, 2006; Huppertz *et al.*, 2006; Lucey and Horne, 2009; Barłowska *et al.*, 2011; Reykdal *et al.*, 2011; Zamberlin *et al.*, 2012; Gaucheron, 2013; Bilandžić *et al.*, 2015). Depending of the cheese type, Na concentration also increases by salting or brine solution. Consequently, the majority of Na remains in whey. This explanation clarifies that two whey samples (WB-3 and WB-4) used for the butter manufacturing probably contain Na from salting or brine solution.

6. CONCLUSIONS

Nowadays, increased consumer demand on milk and milk products increase the number of dairy companies. As a result, vast amounts of dairy wastes are produced every year around the world. Whey is the main waste product of dairy companies from cheese manufacturing, and represents about 85-95% of the milk volume and retains 55% of milk nutrients. Due to high content of organic compounds, whey causes environmental pollution. Nowadays, thanks to advance technologies, whey is converted to several products that possess functional and nutritional properties, such as WPC, WPI, lactose, whey powder.

In this study, butter samples were produced from the whey obtained from the collection centers in four different cities (Uşak, Adana, Burdur and Konya) in Turkey, and assessed their qualities in terms of minor components. Total protein by Kjeldahl, total carotenoid and chlorophyll by UV spectrophotometer, tocopherol isomers by HPLC-Fluorescence detector and minerals by ICP-OES were analyzed in the whey butter samples. For comparison purpose, two commercial unsalted milk butter were analyzed at the same conditions.

The milk butters had substantially greater minor components compared to the whey butters with the exceptions. There was minimal difference in the tocopherol content and lower chlorophyll content. Even there is no specification given by the authorities, this study indicates that milk butter was rich in Ca, P, Mg, K and total carotenoids that are undoubtedly important for human nutrition. It should be kept in mind that milk and whey, as the sources of butters, is appeared to be the cause of these disparities.

Furthermore, the observed variation in the minor components of the whey butters suggests that the whey from four collection centers have different compositions. The differences among the whey sources presented here may mainly be ascribed to the differences in cheese processing and type of cheese produced. It is also worth mentioning that WB-3 and WB-4 had decidedly high content of Na. This is so marked that it would seem to support the Na is virtually through the composition of whey that contains salt or brine solution.

In summary, this study has demonstrated the importance of evaluating minor components in the butter since the source (milk and whey) is of the primary concern. Whey can be used as a source of butter; however, a specific codex apart from the milk butter codex should be composed for the regulatory purpose.

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APPENDIX

Appendix 1. Anova result for protein content in butter samples

Tests of Between-Subjects Effects

Dependent Variable: **Protein**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.144[a]	5	.429	241.592	.000
Intercept	6.142	1	6.142	3461.018	.000
Butter	2.144	5	.429	241.592	.000
Error	.011	6	.002		
Total	8.297	12			
Corrected Total	2.154	11			

[a] R Squared = 0.995 (Adjusted R Squared = 0.991)

Appendix 2. Anova result for carotenoid content in butter samples

Tests of Between-Subjects Effects					
Dependent Variable: **Carotenoid**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.742[a]	5	2.548	881.324	.000
Intercept	42.526	1	42.526	14706.285	.000
Butter	12.742	5	2.548	881.324	.000
Error	.017	6	.003		
Total	55.286	12			
Corrected Total	12.760	11			

[a] R Squared = 0.999 (Adjusted R Squared = 0.998)

Appendix 3. Anova result for chlorophyll content in butter samples

Tests of Between-Subjects Effects

Dependent Variable: **Chlorophyll**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9856.232[a]	5	1971.246	317.461	.000
Intercept	18139.690	1	18139.690	2921.324	.000
Butter	9856.232	5	1971.246	317.461	.000
Error	37.256	6	6.209		
Total	28033.178	12			
Corrected Total	9893.488	11			

[a] R Squared = 0.996 (Adjusted R Squared = 0.993)

Appendix 4. Anova result for tocopherols in butter samples

Tests of Between-Subjects Effects

Dependent Variable: **α-tocopherol**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.676[a]	5	.335	6.639	.020
Intercept	8014.535	1	8014.535	158703.654	.000
Butter	1.676	5	.335	6.639	.020
Error	.303	6	.050		
Total	8016.514	12			
Corrected Total	1.979	11			

[a] R Squared = 0.847 (Adjusted R Squared = 0.719)

Tests of Between-Subjects Effects

Dependent Variable: **β-tocopherol**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.016[a]	5	.003	10.931	.006
Intercept	2.125	1	2.125	7286.429	.000
Butter	.016	5	.003	10.931	.006
Error	.002	6	.000		
Total	2.143	12			
Corrected Total	.018	11			

[a] R Squared = 0.901 (Adjusted R Squared = 0.819)

Tests of Between-Subjects Effects

Dependent Variable: **γ-tocopherol**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.630[a]	5	.126	225.627	.000
Intercept	21.789	1	21.789	39025.209	.000
Butter	.630	5	.126	225.627	.000
Error	.003	6	.001		
Total	22.422	12			
Corrected Total	.633	11			

[a] R Squared = 0.995 (Adjusted R Squared = 0.990)

Appendix 4. *(Cont.)*

Tests of Between-Subjects Effects

Dependent Variable: **Total tocopherol**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.805[a]	5	.761	13.605	.003
Intercept	9148.850	1	9148.850	163567.038	.000
Butter	3.805	5	.761	13.605	.003
Error	.336	6	.056		
Total	9152.990	12			
Corrected Total	4.141	11			

[a] R Squared = 0.919 (Adjusted R Squared = 0.851)

Appendix 5. Anova result for minerals in butter samples

Tests of Between-Subjects Effects

Dependent Variable: **Calcium**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20310.337[a]	5	4062.067	94.216	.000
Intercept	157657.518	1	157657.518	3656.730	.000
Butter	20310.337	5	4062.067	94.216	.000
Error	258.686	6	43.114		
Total	178226.541	12			
Corrected Total	20569.023	11			

[a] R Squared = 0.987 (Adjusted R Squared = 0.977)

Tests of Between-Subjects Effects

Dependent Variable: **Phosphorus**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14738.287[a]	5	2947.657	141.581	.000
Intercept	91701.832	1	91701.832	4404.597	.000
Butter	14738.287	5	2947.657	141.581	.000
Error	124.917	6	20.820		
Total	106565.036	12			
Corrected Total	14863.204	11			

[a] R Squared = 0.992 (Adjusted R Squared = 0.985)

Tests of Between-Subjects Effects

Dependent Variable: **Magnesium**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	160.193[a]	5	32.039	48.543	.000
Intercept	3805.641	1	3805.641	5766.122	.000
Butter	160.193	5	32.039	48.543	.000
Error	3.960	6	.660		
Total	3969.794	12			
Corrected Total	164.153	11			

[a] R Squared = 0.976 (Adjusted R Squared = 0.956)

Appendix 5. *(Cont.)*

Tests of Between-Subjects Effects

Dependent Variable: **Potassium**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25807.664[a]	5	5161.533	99.652	.000
Intercept	220062.917	1	220062.917	4248.691	.000
Butter	25807.664	5	5161.533	99.652	.000
Error	310.773	6	51.795		
Total	246181.353	12			
Corrected Total	26118.436	11			

[a] R Squared = 0.988 (Adjusted R Squared = 0.978)

Tests of Between-Subjects Effects

Dependent Variable: **Sodium**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19859.274[a]	5	3971.855	115.102	.000
Intercept	136324.347	1	136324.347	3950.612	.000
Butter	19859.274	5	3971.855	115.102	.000
Error	207.043	6	34.507		
Total	156390.664	12			
Corrected Total	20066.317	11			

[a] R Squared = 0.990 (Adjusted R Squared = 0.981)

CURRICULUM VITAE

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Education

Master	:	Kahramanmaras Sutcu Imam University, Graduate School of Natural Applied Science, Department of Bioengineering and Sciences, Kahramanmaras, Turkey	2016-2018
Bachelor	:	Polytechnic college, Faculty of Agriculture, Food Industry, Halabjah, Iraq	2010-2014
High School	:	Peshawa, Darbandikhan, Iraq	2005-2009

Language Skill

	English	Turkish	Kurdish	Arabic
Reading :	Very good	Basic	Fluent	Native speaker
Writing :	Good	Basic	Fluent	
Speaking :	Good	Basic	Fluent	

Computer Skill

Advanced knowledge of Microsoft ® Windows environments, Windows Server (2000 and 2003), and Office package (Word, Excel, Access, PowerPoint, FrontPage and Outlook)

Hobbies

Playing soccer, watching soccer, listening to music, reading, hiking, traveling, watching movies, driving.