Edited by MINGRUO GUO

WHEY PROTEIN PRODUCTION, CHEMISTRY, FUNCTIONALITY, AND APPLICATIONS

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Preface

Cheese is a complexed and ancient food that can be traced back thousands of years before modern civilization. It is a major dairy product and plays a very important role in dairy foods evolution and nutrition for the mankind. Cheese making is actually a concentration process of separating casein and fat fractions from milk. Whey is the byproduct from cheese making. Almost every component of whey has been utilized, especially whey protein due to the development of new dairy processing technology.

Whey protein has been considered as one of the most important ingredients of dairy industry because of its high nutrition value and some desirable functionalities. Whey protein is not only used in food applications, but other fields including pharmaceuticals, consumer products, biomaterials, and environmentally safe products.

I have been teaching and doing research on food chemistry and dairy product development for over 30 years. Whey utilization and whey protein functional properties have been one of my top scholarly interests for the past two decades. It is the right time to write a book on this topic to summarize the findings, publications, patents, and unpublished data from my research laboratories. The theme of this work is to discuss the production, chemistry, functionality, and applications of whey proteins. This book includes 10 chapters and covers history and current situation of whey and whey protein production; manufacturing technology of whey protein products; chemistry of whey proteins; denaturation and interactions with other food components; modifications of whey proteins; nutritional properties and applications in functional foods of whey proteins; whey protein functional properties and applications; and use of whey protein in non-food applications.

I sincerely thank all the contributors for their willingness and efforts to collaborate with me on this exciting project.

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1

History of Whey Production and Whey Protein Manufacturing

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1.1 Types of Whey

Milk is a complex of colloidal suspension that is comprised of fat globule, casein micelle colloidal and serum or whey phase (Figure 1.1). Whey (sometimes called milk serum) is a yellowish to greenish clear solution strained from milk curd coagulated by either rennet or acid. Whey components are those small molecules that are not involved in the milk curdling and are able to be strained out. The typical whey solid components include lactose, protein (mainly whey protein), and minerals as illustrated in Table 1.1. Whey liquid contains over 50% of whole milk solids, including the majority of minerals, and nearly all whey proteins and lactose.

Milk coagulated by different method resulted in different types of whey. In general, it can be categorized into sweet whey and acid whey. There is no clear definition between sweet and acid whey, but typically cut off at pH of 5.6. Sweet whey has a pH higher than 5.6, while acid whey is below pH 5.6. Sweet whey is usually from cheese manufacturing (rennet coagulated) and sometimes also called as cheese whey. Acid whey is that from coagulation by fermentation (lactose converted to lactic acid, such as Greek yogurt manufacturing) or by adding acid (acid casein production) (Tunick 2008). The compositional difference between sweet and acid whey is listed in Table 1.2.

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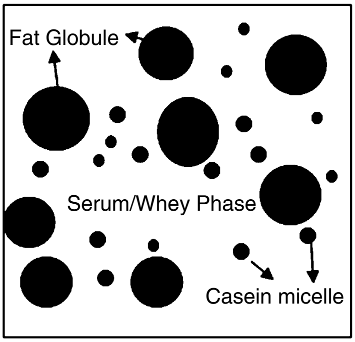

Figure 1.1 Milk is a complex suspension system comprised of fat globules, casein micelles, and the serum/whey phase. Whey proteins, lactose, and minerals are presented in the serum/whey phase.

Table 1.1 Analytical data of whole milk and whey.

Components	Whole milk	Whey
Casein protein (%, w/v)	2.8	<0.1
Whey protein (%, w/v)	0.7	0.7
Fat (%, w/v)	3.7	0.1
Ash (%, w/v)	0.7	0.5
Lactose (%, w/v)	4.9	4.9
Total solids (%, w/v)	12.8	6.3

Source: Data adapted from Smithers (2008).

Table 1.2 Comparison of sweet and acid whey components.

Components	Sweet whey	Acid whey
Protein ($g\,l^{-1}$)	6–10	6–8
Lactose ($g\,l^{-1}$)	46–52	44–46
Minerals ($g\,l^{-1}$)	2.5–4.7	4.3–7.2
pH	>5.6	<5.6

Source: Data adapted from Tunick (2008).

1.1.1 Cheese Whey

Mammals such as cattle, sheep, and goat have been domesticated for over 10 000 years (Clutton-Brock 1999; Beja-Pereira et al. 2006). With the DNA technology, it can be dated back to 17 000 years ago (Troy et al. 2001; Beja-Pereira et al. 2006). Besides milk, cattle and other mammals were also domesticated for traction, wool, or meat. Eastern Asian and Central Africa domesticated cattle as early as other regions, but with no tradition of milking (Clutton-Brock 1999). Until today, people from those regions still have more lactose intolerance than people from other regions like Northern Europe and Near East. The practice of milking a critical step during the prehistoric period because it made a sustainable and nutritious food supply without slaughtering the precious livestock. Making cheese was a milestone of the human civilization history. Cheese, as a preserved food, is much easier to keep than fresh milk. The cheese making during ancient times shares much common as the modern technology, typically including natural fermentation, cooking, straining, and drying.

It is believed that the first cheese was probably produced in a ruminant stomach that is used as a storage vessel for milk (Smithers 2008). The enzyme called rennet naturally presented in the stomach curdled the milk into cheese. The milk curd was further strained to remove the whey. This was probably the first whey disposal even we do not know when and where it took place. The archeological evidence of early milking (usually in the form of a pottery milk/cheese residue) have been disclosed across the world (Evershed et al. 2008; Salque et al. 2013; Scott, Robinson, and Wilbey 1998; Yang et al. 2014). The earliest evidence of cheese making in northern Europe was of the sixth millennium BC (Salque et al. 2013), which is a fragment of a pottery sieve that was used for straining whey (Figure 1.2). The reconstructed sieve vessel (Figure 1.2b) was very similar to the modern cheese sieve from Haute-Loire, France, dating back to the beginning of the twentieth century (Briggs 2012).

A typical cheese production includes a rennet addition to cleave the casein micelle hair (κ-casein hair), thus collapsing the micelle structure and then curdled milk (O'Callaghan et al. 2002). The milk serum phase is able to be strained out by cutting and pressing the milk curd. The rennet coagulation process is

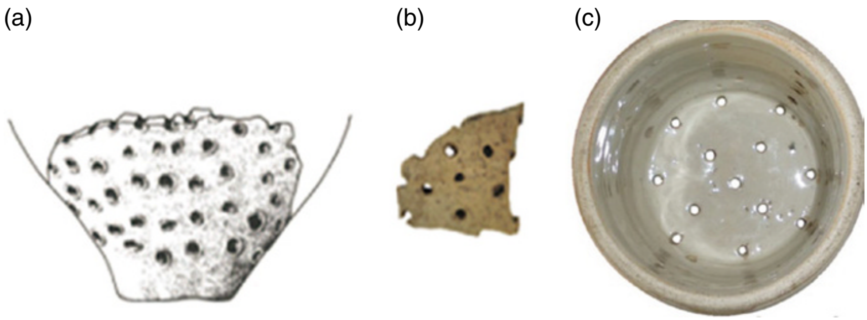

Figure 1.2 (a) The reconstructed sieve vessel; (b) the sieve fragment (7000 years old) found in the region of Kuyavia, Poland; and (c) a modern pottery colander that can be used for cheese straining. *Source:* Modified from Salque et al. (2013).

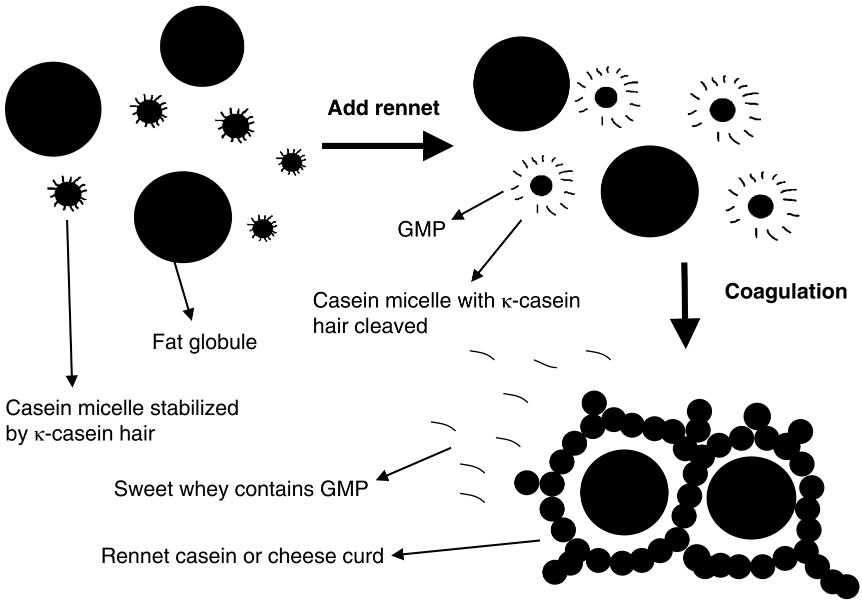

Figure 1.3 Rennet coagulated milk curd and sweet whey.

depicted in Figure 1.3. Rennet is a complex of enzyme produced in ruminant stomachs. The functional component called chymosin is a protease that can cut off the κ-casein hair (a casein protein that stabilizes the micelle structure), thus curdle the casein micelles (Daviau et al. 2000). Fat globules is trapped or emulsified by the casein curd while the serum phase can be strained out which is so called sweet whey or cheese whey. When 1 part of cheese is made, there is 9 parts of liquid whey generated. The κ-casein fragment cut from the micelle by rennet called glycomacropeptide (GMP) (Brody 2000) commonly presented in sweet whey products. Rennet does not work on lactose into lactic acid, therefore, sweet whey has a more neutral pH. Due to the huge volume of cheese making in the world, sweet whey from cheese making is the major commercial available whey today.

1.1.2 Acid Whey

Acid whey is a byproduct of acid coagulated milk including acid casein and Greek yogurt. At neutral pH, casein micelle is stabilized by κ-casein hair (via electrostatic repulsion) and colloidal calcium phosphate (CCP) (de Kruif and Holt 2003). The acid coagulation mechanism is depicted in Figure 1.4. When pH drops, the κ-casein electrostatic repulsion was neutralized and cause the micelle hair layer shrinks (de Kruif 1997). On the other hand, CCP which binds casein

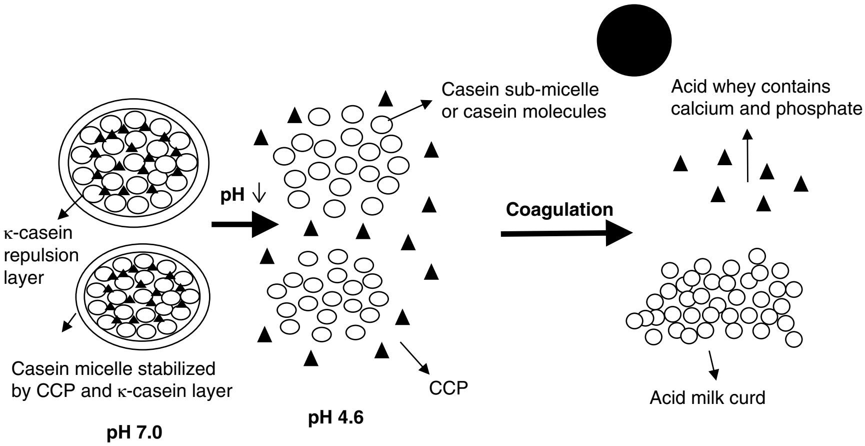

Figure 1.4 Acid coagulated milk curd and acid whey.

molecules is solubilized into the serum phase (Le Graët and Gaucheron 1999). The casein micelle lost it stability, and coagulated into milk curd (Lucey 2003). The whey strained from the acid coagulated milk curd is called acid whey. The acidification can be induced by adding inorganic or organic acid (such as HCl and lactic acid) and/or fermentation (lactose converted to lactic acid).

Due to the different coagulation mechanisms induced by rennet or acid, acid whey and sweet whey demonstrated different physicochemical properties. Besides the pH difference, acid whey typically does not contain GMP (lack of rennet κ-casein cleave), high in ash (calcium released from micelle into serum phase), and perhaps slightly low in lactose (some of lactose converted into lactic acid) compared to sweet whey. For Greek yogurt acid whey, due to the heat treatment before fermentation, some of the whey protein (especially β-lactoglobulin) interact with κ-casein via disulfide-thiol interaction (Lucey 2002; Lu et al. 2013) and become part the milk curd resulting in lower protein content in the acid whey.

1.2 Whey Utilization

Whey was considered as the waste for most of the time, especially when modern industrial mass production of cheese started in the nineteenth century. Whey waste is considered as the most polluted waste of dairy industrial with a biochemical oxygen demand (BOD) between 35 and 45 $kg\,m^{-3}$ and chemical oxygen demand (COD) of 60–70 $kg\,m^{-3}$ (Mawson 1994). Untreated whey has been prohibited from direct disposal in the most regions of the world. Whey is a nutrient dense product, which contained about 50% of milk solids. The history of whey utilization is an excellent example of turning a gutter to a gold (Smithers 2008).

1.2.1 Ancient Wisdom

Our Bronze Age ancestors probably never took the yellow greenish liquid strained from or left in the cheese vessel as a problematic matter at the time when food was precious and nothing could be wasted. The habit of drinking of whey had been passed down all the way to early twentieth century. Even today, whey has been used to make various functional beverages including energy (Singh and Singh 2012), fermented (Pescuma et al. 2010), alcoholic (Dragone et al. 2009), and carbonated beverages (Singh and Singh 2012). As early as 460 BC, the Greek physician Hippocrates prescribed whey to his patients for immune system booster, gastrointestinal ailments and skin conditions (Heffernan 2015; Smithers 2015; Susli 1956). Smithers (2008) mentioned a historic reference of using whey for sepsis, wound healing, and stomach disease in the seventeenth century. Whey consumption became a fashionable habit from seventeenth century in Europe (Holsinger 1978). "Whey house" with a menu of whey porridge, whey soup, whey tea, and whey butter were very popular in Europe from the seventeenth to nineteenth centuries (Smithers 2015). Based on the presumed skin and topical benefits, bathing in cheese whey became popular among the upper class in Europe in the seventeenth to nineteenth century (Heffernan 2015; Smithers 2015). Until today, the Whey spa as a luxury getaway is still attracting thousands of tourists to visit Switzerland and the Alpine regions (Mycek n.d.).

1.2.2 Early Industrial Efforts

Until early twentieth century, the rapid growth of cheese and casein production had driven explosive expansion of whey production. Our forebear's wisdom of using whey was no longer capable to digest the massive amount of whey from cheese industry. Direct disposal of whey to rivers and the environment caused severe pollution and whey became problematic waste streams. Since then, a lot of efforts have been put on by both academia and industry focusing the whey utilization.

Concentrating or drying liquid whey to make it easier to be preserved or transported was one of the early attempts to use whey. One important development came in 1908 when Merrell obtained a sweet whey powder via spray drying (Merrell 1911). In the invention, the liquid whey was introduced through an atomizer and divided into small particles, then dried by so called "moisture absorbing air" in the desiccating chamber. The early industrial whey drying included roller drying (Golding and Rowsell 1932), spray drying (Merrell 1911; Peebles and Manning 1939), and combination of spray and rotary drum drying (Tunick 2008).

The common issue of the early drying technology was the high-energy cost due to the hygroscopic nature of lactose, and whey protein is severe denatured with poor solubility and functionality (Tunick 2008). Multi-stage and vacuum evaporators were developed to improve the solubility (Webb and Whittier 1948; Francis 1969). Because lack of the technology of recovering and purifying whey protein from the liquid, the early industrial whey utilizations were limited on animal feed and applications only relating to lactose's property such as infant food and browning sugar for bakery/confectionary (Berry 1923).

1.2.3 Modern Advancement

The gold mine of whey protein was kept buried until 1970s when membrane filtration technology arrived. Membrane filtration separates components based on particle sizes (Zydney 1998). The details of membrane filtration mechanism will be discussed in Chapter 2. With membrane filtration, the whey protein content in the powder can be increased from 11% (sweet whey powder) up to 90% (whey protein isolate [WPI]). Membrane technology is a non-thermal process which minimizes thermal denaturation of protein. The protein in liquid whey can be recovered by microfiltration or ultrafiltration (Morr and Ha 1993). In order to overcome the defects of whey powder concentrated by evaporation (such as poor solubility and brown color), the modern whey protein concentrate (WPC) and isolate manufacturers use nanofiltration to concentrate the solids prior to spray drying (Atra et al. 2005).

1.3 Major Commercial Available Whey Products

1.3.1 Lactose

Lactose is the most abundant ingredient obtained from whey by crystallization, it is widely used in infant formula, confectionary, bakery, and pharmaceutical products (Holsinger 1988).

1.3.2 Whey Powder

Fresh sweet whey and acid whey can be pasteurized and spray dried to obtain sweet whey and acid whey power. Whey powder contains all the components the fresh whey has, and can be used as milk solid substitute. Whey powder is a good browning ingredient in bakery and confectionary (Dattatreya et al. 2007); however, the application of whey powder is somewhat limited due to its low protein and high ash levels. Demineralized whey is produced by partially remove the minerals by ion exchange, diafiltration or electrodialysis (Houldsworth 1980). The typical levels of demineralization are 25%, 50%, and 90%. Demineralized whey can be used in infant formula, yogurt, and other applications (Jost et al. 1999; Penna, Baruffaldi, and Oliveira 1997; Tratnik and Krsev 1987).

1.3.3 Whey Protein Concentrate (WPC) and Whey Protein Isolate (WPI)

Whey protein is the most valuable component in whey because of its nutritional and functional properties (de Wit 1998; Marshall 2004). Whey protein can be concentrated up to 80% of total solids via ultra-filtration technology. The most common WPC include WPC34, WPC60, and WPC80, which contain 34%, 60%, and 80% of protein, respectively (Table 1.3). WPC34 has very similar composition as skim milk powder in terms of protein, lactose, and fat contents and has been used for skim milk powder substitute for a long time (Hoppe et al. 2008).

With an additional step of micro-filtration, the protein content of WPC80 can be further concentrated to 90% by removing extra fat, which is called WPI. WPI has an exceptional functionality including gelling, emulsifying, and foaming properties (Berry et al. 2009; Gaonkar et al. 2010). WPC80 and WPI can also be instantized for sport and adult protein supplements.

1.3.4 Whey Protein Fraction Products

Different whey protein components have different functional and nutritional properties (Marshall 2004). The details of whey protein separation will be addressed in Chapter 2. Enriched α-lactalbumin, enriched β-lactoglobulin, lactoferrin, lactoperoxidase, and GMP are all available to meet the specific and high end applications.

Table 1.3 Typical composition (%) of major whey products.

	Protein	Lactose	Fat	Ash	Moisture
Whey powder	11.0–14.5	63.0–75.0	1.0–1.5	8.2–8.8	3.5–5.0
Demineralized whey	11.0–15.0	70.0–80.0	0.5–1.8	1.0–7.0	3.0–4.0
WPC34	34.0–36.0	48.0–52.0	3.0–4.5	6.5–8.0	3.0–4.5
WPC60	60.0–62.0	25.0–30.0	1.0–7.0	4.0–6.0	3.0–5.0
WPC80	80.0–82.0	4.0–8.0	4.0–8.0	3.0–4.0	3.5–4.5
WPI	90.0–92.0	0.5–1.0	0.5–1.0	2.0–3.0	4.5
Permeate solids (food grade)	3.0–8.0	65.0–85.0	<1.5	8.0–20.0	3.0–5.0

Source: Data adapted from U.S. Dairy Export Council "Reference manual for U.S. whey and lactose products." Online available at http://usdec.files.cmsplus.com/PDFs/2008ReferenceManuals/Whey_Lactose_Reference_Manual_Complete2_Optimized.pdf.

1.3.5 Milk Mineral Products

Nowadays, every stream of whey has been well utilized including milk mineral products. Permeate from WPC and WPI manufacturing contains mainly lactose and ash (Table 1.3) can be spray dried to permeate solids. Also, lactose can be crystallized from permeate first, and then the de-lactosed permeate containing mainly minerals can be dried, which is called milk salts. Both permeate and milk salts are excellent source of minerals and can be used for feed and food ingredients.

1.4 Summary

Whey is the yellowish to greenish clear solution strained from milk curd coagulated by either rennet or acid. Whey has been used for thousands years such as drinking. With the increasing amount of whey generated from cheese industry and the development of technology, a lot of efforts have been put on by both academia and industry focusing the whey utilization. Nowadays, every stream of whey has been well utilized including lactose, whey protein and milk mineral products.

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2

Manufacturing Technologies of Whey Protein Products

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Liquid whey or whey waste separated from cheese or other milk curd is a diluted liquid which contains 3.3–6.0% lactose, 0.32–0.7% protein, and 0.5–0.7% minerals (Marwaha and Kennedy 1988). The majority of dry matter in liquid whey is lactose, ash, and a low level of protein. The excellence of whey protein functionality only exhibits when the protein is concentrated and purified. In another word, how much whey is utilized depends on how much whey protein can be recovered, concentrated, and purified from the dilute liquid whey. In early ages, the whey can be only used for animal feed, land fertilizer, and such low value added applications due to technology restriction to recover and concentrate the proteins. The whey protein boom did not start until the advances of the technology of spray dry, and membrane filtration. The wide use of membrane filtration in whey industry turn this problematic whey waste into a gold mine because it is able to extract whey protein with minimum changes to the protein molecules. Furthermore, whey protein is a group of proteins of different properties, functionalities, and nutritional benefits. Further whey protein fractionation, such as making β-LG or α-LA enriched whey protein concentrate (WPC), is the new trend to maximize and expand the value and application of whey protein. This chapter will review technology of how to recover and fractionate whey protein as well as the current process to make commercial whey protein products.

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