ABSTRACT

DEVELOPMENT OF A BEVERAGE FROM WHEY

Whey is a byproduct of cheese manufacture. Although highly nutritious, every year millions of tons of whey are discarded as waste. There are several uses that whey is put to, such as cattle feed, fertilizer, and in the food industry as a protein source, after sufficient treatment. These treatment methods are expensive, but necessary, as whole whey by itself carries an unpleasant cheesy taste and aroma. Whey was treated with activated carbon and then formulated into a suitable beverage with a suitable stabilizer. Activated carbon treatment was successful in marginalizing the color and odor. Color was reduced from opaque to nearly colorless by 81.63%, and the beverage had a desirable viscosity that remained even after heat treatment (70°C). Sensory evaluation studies showed a definitive amiability for the beverage among the subjects tested.

Akshay Aswani
December 2010
DEVELOPMENT OF A BEVERAGE FROM WHEY

by

Akshay Aswani

A thesis
submitted in partial
fulfillment of the requirements for the degree of
Master of Science in Food and Nutritional Sciences
in the Jordan College of Agricultural Sciences and Technology
California State University, Fresno
December 2010
APPROVED

For the Department of Food Science and Nutrition:

We, the undersigned, certify that the thesis of the following student meets the required standards of scholarship, format, and style of the university and the student's graduate degree program for the awarding of the master's degree.

_________________________
Akshay Aswani
Thesis Author

_________________________
Gour Choudhury (Chair)  Food Science and Nutrition

_________________________
Erin Dormedy  Food Science and Nutrition

_________________________
Dennis Ferris  Food Science and Nutrition

For the University Graduate Committee:

_________________________
Dean, Division of Graduate Studies
AUTHORIZATION FOR REPRODUCTION
OF MASTER’S THESIS

___X______ I grant permission for the reproduction of this thesis in part or in its entirety without further authorization from me, on the condition that the person or agency requesting reproduction absorbs the cost and provides proper acknowledgment of authorship.

___________ Permission to reproduce this thesis in part or in its entirety must be obtained from me.

Signature of thesis author: ________________________________
ACKNOWLEDGMENTS

When I started this research project in 2005, I conversed with Dr. Choudhury about my intentions of culminating a nutritive sports beverage that would be easily available and affordable. His creative rendering paved the way for what this beverage has come to be. Since that initial idea, this “product” has been shifted, drifted, stalled, and crawled eventually with the fruition of a successful endeavor that started with one boy’s simple pursuit. I would like to take this opportunity to thank Dr. Choudhury for his time rendered patience and expertise. This thesis would not have taken the shape it has sans his visionary outlook. I would also like to thank Dr. Dormedy for her support and her faith in me and my abilities. Dr. Ferris, in spite of his busy schedules and my misgivings with meeting deadlines, was unremittingly forgiving and very helpful, understanding the specificity of my requirements from him.

I would also like to thank my fellow students, Preetam Sarkar, Sourabh Parulekar, Shanaz Ahmed, Freddie Pacudan, and Nikhil Setia for their support and encouragement, and Daniel Avila from Dairy Processing, who has been extremely cordial and helpful.

Lastly, I cannot put into words how grateful I am to my parents, Laju and Kishin Aswani, for all the sacrifices they have made to make this goal of mine a tangible reality.
TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION ............................................................................ 1

CHAPTER 2: LITERATURE REVIEW ................................................................. 4
  Milk and Whey .................................................................................................. 4
  Benefits of Whey ............................................................................................... 5
  Nutritional Benefits of Major Whey Minerals .................................................. 7
  Whey Utilization ............................................................................................... 9
  Research Goal and Objectives ........................................................................ 16

CHAPTER 3: MATERIALS AND METHODS .................................................... 17
  Materials .......................................................................................................... 17
  Equipment ....................................................................................................... 17
  Methodology ................................................................................................... 17
  Market Survey ................................................................................................. 21
  Stabilization and Viscosity Studies ................................................................ 22
  Stabilizer’s Effect on Post Pasteurization Precipitation of Whey Protein ...... 22
  Formulation ..................................................................................................... 23
  Sensory Evaluation of the Final Formulation ................................................. 23

CHAPTER 4: RESULTS AND DISCUSSION ..................................................... 26
  Whey Refining by Charcoal Adsorption ......................................................... 26
  Market Survey ................................................................................................. 29
  Stabilization and Viscosity Studies ................................................................. 30
  Formulation ..................................................................................................... 30
  Sensory Evaluation for Finished Beverage ..................................................... 32

CHAPTER 5: CONCLUSION ............................................................................... 33
LIST OF TABLES

Table 1 – Whey Composition ........................................................................ 5

Table 2 – Time and Concentration Variables in Charcoal Adsorption Study ........................................ 20
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Jacketed reaction flask with water bath: RTE 750</td>
<td>18</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Effect of temperature on charcoal adsorption</td>
<td>27</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Effect of charcoal concentration on color removal</td>
<td>29</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Comparative analysis of the effects stabilizers on the precipitation of proteins in whey</td>
<td>31</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Comparison of precipitate with whey blended with stabilizer (right), and plain whey (left), centrifuged after storage for 72 h.</td>
<td>31</td>
</tr>
</tbody>
</table>
CHAPTER 1: INTRODUCTION

Whey, extracted from milk, is a wholesome protein source known to be “high quality protein,” as it contains all nine of the essential amino acids. Whey proteins are especially high in essential amino acids such as tryptophan, which helps enhance brain serotonin levels (Delgado-Andrade and others 2006). Whey is not only a good source of protein, but is also significantly rich in minerals and lactose. Whey and casein are the two proteins present in milk. During manufacture of cheese, the casein coagulates to form the cheese curd. The milk fats get entrapped within the casein complex, allowing the rest of the milk components to float to the surface. This includes the whey proteins, minerals, and lactose, which are essentially the by-product of cheese manufacture. Although the whey that is sloughed out is 90% water and only 0.6% of it is actually protein, processes such as ultra filtration and ultracentrifugation convert whole liquid whey to whey concentrate, which is about 90% protein (Huffman 1996). Yet, most of the whey produced every year is disposed of into rivers and lakes. Currently, the world produces well over 80 x 10^9 L of whey per year (Smithers and others 1996). During cheese manufacture, for every 10 lb of milk used, only 1 lb is converted to cheese, and 9 lb is sloughed off as whey (Bhattacharjee and others 2006). Due to the biological oxygen demand (B.O.D.), the discarded whey is considered “sewage,” and hence has to undergo appropriate treatment methods before being dumped into the ocean as waste, which is an additional cost to the manufacturing company. Alternative means of utilization of whey have been developed over the past few years. Whey protein, as a whole, has been used as a component for functional foods. The α-lactoalbumen and β-lactoglobulin fractions have been extracted from whey and used as functional food ingredients. Whey is also used as
animal feed or as a fertilizer (Olson 2003). But the largest utilization of whey is as protein supplements, some that are specifically designed for athletes as well as meal replacement shakes and protein bars.

Many studies have been carried out to determine whether athletes have a higher protein demand in their diets. Protein requirement is determined by calculating nitrogen balance. For an average person, the daily dietary intake of protein is 0.8 g per kilogram of body weight. Studies done by Tarnopolsky and others (1992), and Phillips and others (1993) show that resistance training and endurance athletes do have a higher protein requirement. This experimentation was carried out by feeding athletes different amounts of protein and calculating their nitrogen balance (Tipton and Wolfe 2004). It is hard to advocate a general guideline for protein intake. The requirement for a marathon runner would obviously vary from that of a weightlifter or a football player. The protein Daily Recommended Intake (DRI) of strength training athletes sometimes could go up to as high as 2.5-3.0 grams per kilogram of body weight. Such high intakes are sometimes hard to achieve from a regular diet, and hence there is such a huge market for protein supplements. As sporting events get even more competitive, every small aspect of the athlete’s training regime is essential in overall performance, and maintaining a proper diet is one of the essential parameters. The right amount of protein supplementation helps repair tissue faster and provides an increase in muscle mass, giving the athlete a competitive edge.

Dietary supplements sales in a year are in excess of $3.2 billion (Anon 1998). Whey protein products are a growing segment of the supplement industry, increasing as newer methods of product recovery and manufacture are being developed. Most protein shakes are manufactured and sold in powder form, after ultra filtration and spray drying of the whole whey. This is an expensive process,
and the high price of whey-based products reflects this expense. If a novel method was developed whereby whole whey could be utilized as a beverage without being subject to these treatments, whey-based beverages could be produced at a much lowered manufacturing cost price, which would benefit the manufacturer, as well as the consumer. The only constraint with using whole whey in a beverage as opposed to micro-filtered, ultra-centrifuged whey permeate is the unpleasant cheesy taste and odor. The causative agent(s) of these characteristics is unknown, but some purification studies using the process of charcoal adsorption has found to reduce surface contaminants of whey proteins (Cornec and Narsimhan 1998). This study is an attempt to develop a beverage from whey that is nutritious as well as palatable at minimal cost by reducing processing costs.
CHAPTER 2: LITERATURE REVIEW

Whey is a nutritive dairy byproduct, replete in proteins and minerals. It has found use in several facets of the food, beverage, and agricultural industries. There still is room for more innovative utilization of whey in the beverage industry.

**Milk and Whey**

Milk is a complex colloidal solution composed of 87.5% water, 3.9% fat, 3.4% protein, 4.8% lactose and 0.8% minerals. Milk proteins are of two types, caseins and whey proteins. Caseins are the dominant protein at 80%. During the process of cheese manufacture, casein coagulates to form the cheese curd, trapping the milk fats within the casein complex. The serum containing lactose, whey proteins, minerals and water is separated and is collectively referred to as “whey.” Whole whey is approximately 93% water and 0.6% protein. The composition of whey is presented in Table 1. Whey proteins are mainly comprised of $\alpha$-lactalbumen and $\beta$-lactoglobulin but also include bovine serum albumin, lactoferrin, lactoperoxidase enzymes and glycomacropeptides (Driskell 2007). Immunoglobulins are also a protein component of whey, but they exist in very small quantities (Bylund 1995). Whey proteins are very high in essential and branched chain amino acids, which are necessary for serum replenishment during exercise and the anabolic period post-exercise and are also easily soluble at the pH of the digestive tract. As compared to other proteins that need to be “pre-digested” to be soluble in the gut, whey protein ingestion quickly spikes blood amino acid levels in a much shorter period of time after ingestion (Bounous and others 1991).
Table 1 – Whey Composition

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Amount (%w/w) in:quirel Whey</th>
<th>Acid Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>93-94</td>
<td>94-95</td>
</tr>
<tr>
<td>Dry Matter</td>
<td>6-6.5</td>
<td>5-6</td>
</tr>
<tr>
<td>Lactose</td>
<td>4-5.5</td>
<td>3.8-4.3</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>Traces</td>
<td>≤ 0.8</td>
</tr>
<tr>
<td>Total Protein</td>
<td>0.8 – 1</td>
<td>0.8 – 1</td>
</tr>
<tr>
<td>Whey Protein</td>
<td>0.6 – 0.65</td>
<td>0.6 – 0.65</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Minerals</td>
<td>0.5-0.7</td>
<td>0.5-0.7</td>
</tr>
</tbody>
</table>


Benefits of Whey

Muscles are primarily made of proteins. Proteins are responsible for creating most of the structural adaptations that make muscles bigger, stronger, faster, and more fatigue-resistant in response to exercise. By supplementing the diet with whey protein, one can accelerate and enhance these beneficial changes in muscle structure and also boost heart and immune health (Kinsella and Whitehead 1989).

Nutritional Benefits of Whey Proteins

Whey protein supplements have shown to enhance muscle building and fat burning effects of exercise. They also improve performance in workouts, speed recovery and may even reduce the chances of injury (Papenburg and others 1990).
The benefits of whey protein have been demonstrated in a number of studies, including a study published in the *Advanced Food Nutrition Research*, which looked at the effects of whey protein supplementation on muscle growth in men undertaking a 6-wk resistance training program. In this study, subjects combining resistance training with whey protein supplementation increased their lean muscle mass and strength significantly more than subjects who completed the same exercise program without supplementation (Kinsella and Whitehead 1989).

Every hard workout causes microscopic damage to muscle fibers. One of the symptoms of this damage is a type of muscle pain known as DOMS (delayed-onset muscle soreness). The faster the body can repair this damage and reduce the associated soreness, the better will be its ability to perform in the next workout (Kinsella and Whitehead 1989). This particular benefit of whey protein was demonstrated in a study from Vanderbilt University that involved Marine recruits as subjects. During boot camp, these subjects were randomly assigned to receive either a recovery supplement containing whey protein or a placebo after physical training each day. By the end of boot camp, those Marines who had received the recovery supplement with whey protein had experienced 37% fewer joint and muscle injuries than the Marines in the placebo group (Kinsella and Whitehead 1989).

Whey proteins are advantageous not only to athletes but to the general population as well. They are beneficial for physically active individuals to repair and rejuvenate muscle fibers, help maintain body weight and keep the heart and immune system healthy. Adequate consumption of whey protein assists in maintaining body weight as whey proteins play a role in balancing blood sugar levels. An individual with normal blood sugar levels has a greater rate of fat loss, more energy, and lesser tendency to overeat. Research has also shown that whey
protein is good for the heart, helps fight cancer, and also improves the immune system of people suffering from AIDS (Kinsella and Whitehead 1989). In addition, inclusion of whey proteins in the diet has also shown to reduce cholesterol (Kinsella and Whitehead 1989) and helps maintain strong bones and muscles during aging. In fact, one study showed that older men who ate whey protein regularly had an increase in protein synthesis that helps limit muscle loss over time. The same study showed that elderly people who consumed lower amounts of protein had a loss of bone density particularly in the hip and spine. Research indicates that whey protein can help reduce the amount of bone density loss. In a study conducted in 2000 by Hakkak and others at the University of Arkansas, whey and soy-rich diets were shown to protect against mammary tumors in rats. According to the study, soy protein in the diet reduced tumor incidence by 20%, and whey proteins were shown to be twice as effective as soy (Hakkak and others 2000). In short, whey protein consumption is beneficial irrespective of age and fitness goals.

Nutritional Benefits of Major Whey Minerals

Whey contains several minerals with magnesium, calcium, and phosphorus being the most prominent.

Magnesium

Magnesium is the fourth most common cation found in the human body and is an essential co-factor in over 300 enzymatic reactions (Fawcett and others 1999). It is mainly found in bone (53%). Intracellular muscles contain 27%, and 19% of the body’s magnesium is found in soft tissues leaving less than 1% in serum and red blood cells (Elin 1994). Amongst other functional properties such as hormonal receptor binding, transmembrane ion flux, neurotransmitter release,
control of cardiac excitability, and vasomotor tone, magnesium plays a major role in exercise physiology. This includes muscle contraction, regulation of adenylate cyclase (Adenyline Triphosphate synthesizing enzyme), and synthesis of carbohydrates as well as nucleic acids (including proteins that are required for muscle restoration) (Fawcett and others 1999). Some of the enzymatic reactions catalyzed by magnesium involve energy synthesis, making magnesium an essential mineral for athletes. Low magnesium levels during endurance-type activities could prove detrimental and athletes competing with low magnesium levels could suffer from severe muscle spasms causing cramps (Rosenbloom 2000). The daily estimated average requirement for magnesium is 200 mg in females and 250 mgs in males (Martindale and Reynolds 1996).

Calcium

The fact that 99% of the body’s calcium is stored in the bones and teeth (Rosenbloom 2000) illustrates its importance in bone structure and formation. Calcium is responsible for bone metabolism, blood coagulation, nerve impulse transmission, cellular adhesiveness, and neuromuscular excitability aside from being involved as a cofactor in several enzymatic reactions and hormone release (Olsen 2003). The serum level of calcium is maintained at 2.2 to 2.5 millimoles/L (Olsen 2003); however, the detrimental effects of calcium deficiency have not yet been pinpointed due to its slow turnover and the detrimental effects are too gradual to be effectively demonstrated in humans (Nordin 2000). Studies on animals have shown a direct correlation on deprivation of calcium and onset of osteoporosis (Nordin 1960). This can be attributed to reduced calcium absorption and increased calcium re-absorption due to an effect of estrogen on bone, especially in post-menopausal women (Nordin and others 1999). Also, athletes
have a higher calcium requirement, due to loss in sweat during exercise (Rosenbloom 2000). Recent studies have also shown that increase in muscle mass from physical activity (weight training as well as various day-to-day activity) is followed by an increase in skeletal growth. This increase in skeletal mass requires additional calcium intake (Anderson 2000).

Potassium

Potassium is one of the four minerals essential for cell functionality. It is an ionic salt and is similar to sodium in structure. Potassium naturally occurs in the diet in meats, fish, dairy, fresh fruits, and vegetables. Potassium intake is essential for maintaining normal heart health. Its main functionality in the human body is in maintaining osmotic balance between cells and interstitial fluids and in facilitating neural transmissions in the brain (Hellgren and others 2006). Potassium is an integral cofactor in many major biochemical functions of the body, thus maintaining osmotic and electrolytic balance and preventing involuntary muscle contraction (that could lead to cramping), and is thus necessary to be constantly replenished in athletes, as salts such as potassium are lost in heavy amounts in sweat and urine (Olsen 2003).

Whey Utilization

For every 10 lb of milk used for manufacturing cheese, only 1 lb is converted to cheese and the remaining nine pounds is discarded as whey (Bhattacharjee and others 2006). Due to the high biological oxygen demand, and its impact to the environment, the discarded whey is considered as “sewage” and hence has to undergo appropriate treatment methods before being dumped into the ocean. These treatment methods could add millions of dollars to the production costs, as well over $80 \times 10^9$ liters of whey is produced per year (Smithers and
others 1996. Only a small percentage of this whey is utilized as a byproduct and most of it is considered waste.

Whey Utilization in Food Products

Alternative means of utilization of whey have surfaced over the past few decades. Because of high transportation cost and susceptibility to deterioration during storage, fresh pasteurized liquid whey is rarely used as much for food, but is rather concentrated by evaporation, reverse osmosis, or ultrafiltration to condensed products or maximally concentrated by drying. Whey powder, whey protein concentrates, whey protein isolates, reduced-lactose whey, and demineralized whey are all derived from whole whey. Whey protein concentrates (WPCs) are available with protein levels typically ranging from 34% to 85%. WPCs also contain lactose, fats, and minerals. With higher protein contents, the percentages of lactose in whey protein concentrates are lower. Whey protein isolates contain at least 90% protein on a dry weight basis and little, if any, lactose or fat (Huffman 1996). In addition, whey protein isolates can be heated with acid or treated with proteolytic enzymes to form hydrolyzed whey proteins. As a result of new technologies, a variety of biologically active amino acids, peptides, and protein fractions can be isolated from whey protein (Papenburg and others 1990).

Foods as diverse as breads, processed meats, dried soups, surimi, whipped toppings, and confectionaries each can benefit from a broad range of functions that dairy proteins have to offer. For example, whey protein concentrates form gels useful for meats, as well as custards, puddings, and confectionaries. Certain formulations possess good whipping and foaming properties for use in whipped toppings. Whey proteins can also stabilize a variety of food emulsions, including salad dressings, soups, and processed meats. Today, whey proteins are used in the
baking industry as nutritious moisture binders. The main protein found in whey, beta lactoglobulin, shows a high capacity to bind flavors. Researchers at Cornell University suggest that food processors may need to add additional flavors to overcome this binding where high levels of beta lactoglobulin are present (Kinsella and Whitehead 1989). Whey as a whole is also used as a component for functional foods. Alpha-lactoalbumen and Beta-lactoglobulin have been extracted and used as functional food ingredients. Although some amount of excess whey is also used as animal feed or as fertilizer (Olson 2003), a significant amount of utilization of whey is in protein supplements. Some of these supplements are specifically designed for athletes as well as meal replacement shakes and protein bars.

**Whey Utilization in Beverages**

Ready-to-drink (RTD) beverages being marketed to the widest possible consumer base often incorporate some type of protein fortification. For this purpose, whey proteins are frequently an ingredient of choice. High-protein drinks, typically greater than 4% protein, generally are ingested for amino acid replenishment and anabolic muscle-building during and after exercise. Low-protein drinks that are typically less than 4% protein are targeted to young children as a healthy alternative to carbonated sugary beverages (Wong and Watson 1995).

Whey-based beverages have been in existence for a long time since Graeff first patented a simple procedure by heating, de-aerating, and charging whole whey with carbon dioxide and formaldehyde in 1898. Since then, there have been several different types of whey-based beverages ranging from fermented and alcoholic drinks to beverages with varied treatment methods and additives.
Considerable research literature on whey-based beverages has been published in Germany and Eastern Europe (Holsinger and others 1974).

Several countries have researched the use of de-proteinized whey as a beverage base. The whey is either subject to high temperatures (about 90 °C) or proteolytic enzymes to coagulate the proteins and separate them by filtration or centrifugation. The supernatant then forms a base that could be either treated with herbal extracts or other additives, or fermented and carbonated (Holsinger and others 1974). Several processes have been developed in order to utilize whole whey without fermentation, such as heat coagulation, condensation (Mauroy 1960) and addition of preservatives such as acids and pasteurized sugars (Holsinger and others 1974). A beverage called “Detskii” was developed in Russia in the 1960s that had a lengthened shelf life due to the addition of pasteurized sugar syrup and carrot juice to deproteinized whey (Kustovskaya 1969). Many others have created beverages by deproteinizing whey and in some cases also adding carbonation. Rivella is one such sparkling drink created in Switzerland in 1952. It is produced by fermenting deproteinized whey with lactic acid bacteria condensing, filtrating, carbonating, and then flavoring the clear liquid (Holsinger and others 1974). Although the fermentation creates trace amounts of alcohol, it is still considered a non-alcoholic beverage. Since then, the Polish have developed a number of fermented non-alcoholic whey beverages, such as whey champagne and whey *kwasi*—a caramel-colored beverage similar to beer (Lang and Lang 1971). Several other researchers started looking toward whey as a substitute for malt in brewing, especially during World War II when there was a shortage of ingredient and raw material supply. Whey served as an ideal substitute not only because it was easily accessible but also because it has properties that are suitable for brewing. Whey has high salt content, an excellent ability to bind to carbonic acid and on
prolonged heating under the right pressure certain constituents of whey develop
caramel like flavor similar to that of malt from barley. For a good brew however, a
maximum of 30% malt can be replaced with whey (deproteinized) in order to keep
the pH in between 4.5-5.5 as whey has a high buffering capacity (Roeder 1939). A
whey malt beer with up to 50% substitution with whey can be achieved with re-
fermentation with sugars. With the use of lactose fermenting micro-organisms,
beer can be made without using any other form of malt. Whey can be used
extensively in beer manufacture with different micro-organisms, varied
concentrations, temperatures, fermentation duration, and procedures (Hesse 1948).
Aside from beer, whey has also been found useful in the production of wine and
other alcoholic beverages by increasing the ethyl alcohol yield from improvised
fermentation techniques. Yoo and Mattick (1969) discovered that maximum ethyl
alcohol can be obtained by fermenting whey with 12% lactose concentration.
Induced lactic acid production and carbonation has also helped create sparkling
wines from whey (Holsinger and others 1974).

Fermented whey has also been used in non-alcoholic beverages. Schulz,
during World War II, developed Lactrone, an acid whey concentrate containing
17% total solids, 10% lactic acid, 2% protein 2% reducing sugars, and 3% ash
(Schulz 1947). Schulz and Draeke (1947) used fermentation and vacuum
evaporation in order to expunge the cheesy whey odor, and then used the product
left after distillation as a diluted beverage. They mixed in fruit juices especially
from citrus fruits, as citrus fruits have an aroma and taste that is compatible with
whey, particularly acid whey. Several whey-based drinks have been developed
with citrus fruits such as oranges, grapefruits, etc. (Holsinger and others 1974). O-
way, a product combining acid whey and orange juice was developed at Michigan
State University in 1969 containing 0.7 to 1% protein (Brunner and others 1969).
Way-Mil, also developed at Michigan State University, created a new trend by using whey as a milk imitation product by adding fat in the form of vegetable oils (Hollsinger and others 1974). Edmonson and others (1968) developed a similar product with whey and cream, which when pasteurized and flavored with chocolate scored almost as high on a 9-point hedonic scale as commercially available chocolate milks (6.5 in comparison to 6.9). Whey has also been used as a milk substitute in flavored fruit drinks with different combinations of cream, sugar, and stabilizers making it a lighter yet nutritionally dense beverage. With newer technologies such as electrodialysis, gel permeation, membrane filtration, ultrafiltration, and combinations of these methods, protein isolates of higher concentrations can now be manufactured and used to fortify pre-existing beverages with little or no change in taste or flavor. Additional components to add flavor can be added to whole or isolated whey in the form of artificial flavors or natural adjuncts such as fruit juices and extracts (Hollsinger and others 1974).

The inclusion of fruit extracts in a beverage would possibly increase healthful benefits. Recent studies indicate that phytonutrients found in fruit possess several beneficial properties; the anti-aging power of blueberries, the joint pain relieving properties of cherries, and a whole host of powerful properties in pomegranates are just a few examples (Nordin 2000).

Exercise increases oxygen utilization, which leads to the formation of free radicals. These free radicals are responsible for cellular damage closely linked with aging and cancer. Fruits such as berries, peaches, plums, and cherries are very rich sources of antioxidants that diminish these free radicals. Hence, the inclusion of fruit in a whey beverage not only increases flavor and nutrient density but also helps protect the body from free radical damage and from aging and cancer in the long run.
However, fruit and dairy mix beverages have not yet gained as much popularity here as compared to Europe. Whey drinks are commonly found on shelves in the European market. Danone, a French company, markets “Frumixx,” a beverage made from fermented milk, whey, and 6% fruit juice targeted at children. Another Austrian drink called Frisch and Frucht, contains probiotic whey and fruit juice and is fortified with multivitamins and calcium. This beverage is distributed by Nom AG, and now goes under the label of simply “Fresh.” Muller markets several whey-based beverages across Europe including “Froop” in Germany and Juicer Muller in the Netherlands (Berry 2005).

In the United States, soy is still a preferred ingredient in ready to drink protein beverages, such as in the “super protein” range of drinks produced by Odwalla. Whey is beginning to be used in beverages, especially as a probiotic. Yoplait nouriche, for example, is made from yogurt and fermented whey. Starbucks is planning to soon unveil a new beverage made from fruit juice and whey powder. This is already being tested in some markets and is in initial stages of development. Powdered whey is easily transportable and can be isolated to high protein concentrations (up to 90%) and hence is the preferred choice for most whey products including RTDs (ready to drink beverages). However, the processing techniques employed to obtain powdered whey such as ultracentrifugation, microfiltration, etc., are time-consuming, tedious, and expensive. Concentrated whey powders for this reason are a relatively expensive ingredient. In case of RTDs, the powdered whey is re-mixed with water, which makes the entire process redundant. Using whole whey directly in a ready to drink beverage would greatly reduce cost of production.
**Research Goal and Objectives**

Two main constraints in using whole whey are odor and palatability and protein precipitation. Whole whey has a distinctive cheesy flavor that makes whey unpalatable. This flavor does not blend with fruity flavor and hence needs to be eliminated. The $\alpha$-lactalbumen and $\beta$-lactoglobulin denature when subjected to temperatures higher than 71.9°C (Boye and Alli 2000). During pasteurization, the whey is heated to 72°C causing the proteins to denature, and precipitate out of the solution. This is undesirable in a beverage as the precipitate would form clumps.

The overall goal of this study is to utilize existing body of knowledge to formulate a desirable beverage from whey. The specific objectives are:

1. Minimizing cheesy odor and color from whole whey.
2. Stabilization of whey proteins in order to prevent them from precipitating out of solution.

Formulation of a beverage with acceptable viscosity, palatability, and appearance.
CHAPTER 3: MATERIALS AND METHODS

**Materials**

Whey was obtained from the Dairy Processing Unit at California State University, Fresno during mozzarella cheese processing. The whey was collected before the salt addition step. Food grade activated carbon was obtained from Mallinckrodt, Hazelwood, MO. Food grade stabilizers (TIC Pretested® Freedom Gum X-PGA/LV Powder and TIC Pretested® Dairyblend 603-EP FF) were obtained from TIC gums (Atlanta, GA). Natural food flavoring was donated by Flavopro, Bakersfield, CA. Fruit juice concentrates and fresh fruit was purchased from Save Mart Supermarket (Fresno, CA) and strawberries obtained from Wawona Frozen Foods (Fresno, CA).

**Equipment**

A jacketed reaction flask was custom designed by Kimble Kontes (Vineland, NJ) for our research. A water bath (RTE 750) was purchased from Thermo electron corporation USA (Figure 1). A Brookfield Viscometer manufactured by Brookfield Engineering Laboratories (Middlebro, MA), a centrifuge (Accuspin 400) purchased from Fisher Scientific (Pittsburgh, PA), and a UV Spectrophotometer manufactured by Thermo Electron Corporation (Madison, WI) were used for adsorption studies.

**Methodology**

The whey was first treated with activated carbon to check for efficacy of color and odor removal. The next step was adding stabilizer and testing for precipitation, and finally formulating the beverage with flavors and adjuncts.
Whey Refining by Charcoal Adsorption

It has been demonstrated by Jolles in 1913 that activated carbon has strong adsorption ability. Fine particle size increases surface area and activation by acidulation and steam treatment helps increase surface adsorption. The dynamics of surface contaminant adsorption of whey proteins by activated carbon were studied by Cornec and Narsimhan in 1998. Native and charcoal-treated whey proteins were compared via various testing parameters to study the adsorption characteristics. Thin Layer Chromatography showed that the surface contaminants adsorbed by the carbon were triglycerides and free fatty acids. Also, the charcoal treatment does not affect the protein structures (Cornec and Narsimhan 1998). The carbon can then easily be filtered out of solution once the odor and color compounds have been adsorbed (Jolles 1913).
The whey was treated with food grade activated carbon at varied temperatures, concentrations, and time intervals to determine optimum treatment conditions. Each sample was treated in the reaction flask and temperature was regulated by a water bath. The samples were stirred with a magnetic stirrer at 350 rpm.

An absorption spectrum was run for untreated whey on a UV spectrophotometer and the spectrum peaked at 465 nm. Thus absorbance of each sample (control and charcoal treated) was measured at 465 nm. Color removal was calculated indirectly on a percentage basis using formula (1). Measurements and calculations were repeated for whey samples at varying temperatures, time intervals, and concentrations. The results were then analyzed on SPSS (version 16.0, Microsoft Inc. USA) to determine efficacy of the treatment.

\[
\text{Percentage Color Removal} = \frac{A_{UT} - A_T}{A_{UT}} \times 100 \hspace{1cm} (1)
\]

Where: \( A_{UT} \) is the absorbance of untreated whey sample

\( A_T \) is the absorbance of whey sample after charcoal treatment

**Effect of temperature on charcoal adsorption.** To measure the effect of temperature on charcoal adsorption, samples of whey were treated with activated carbon at different temperatures. Each sample had 0.5 g of charcoal mixed into 100 mL of whey in a jacketed reaction flask attached to a water bath to ensure temperature regulation. The samples were stirred with a magnetic stirrer at 350 rpm for 15 min. The samples were subjected to room temperature (25°C), 40°C, 50°C and 60°C. Three trials at each temperature were performed and percentage clearance was calculated using the aforementioned formula.
Effect of charcoal concentration and absorption time on refining of whey.
Five different concentrations of charcoal were tested (0%, 0.5%, 1%, 1.5%, and 2%). Each of these concentrations was tested at four different time intervals (0 min, 5 min, 10 min, and 15 min). All samples were stirred at 350 rpm with a magnetic stirrer. Three trials were conducted (Table 2) and percentage clearance was calculated and the subsequent data were subjected to statistical analysis using SPSS (version 16.0), with Tukey’s post hoc test.

Table 2 – Time and Concentration Variables in Charcoal Adsorption Study

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Concentration in whey (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>✓      ✓   ✓   ✓   ✓   ✓</td>
</tr>
<tr>
<td>5</td>
<td>✓      ✓   ✓   ✓   ✓   ✓</td>
</tr>
<tr>
<td>10</td>
<td>✓      ✓   ✓   ✓   ✓   ✓</td>
</tr>
<tr>
<td>15</td>
<td>✓      ✓   ✓   ✓   ✓   ✓</td>
</tr>
</tbody>
</table>

Sensory evaluation to determine efficacy of charcoal adsorption. The two samples tested for difference were untreated whey and whey after charcoal adsorption using Triangle Test. Samples were served in black 2 oz cups. The
samples were prepared in two sets of three, with two cups containing one sample and the third containing the other. All three cups were numbered with random 3 digit numbers not ending in 0. Six possible combinations were prepared (316, 641, 380, 813, 231, and 661). Numbers were randomly selected from the random number selection table (Meilgaard and others 2007). These were randomly arranged so as to avoid discrepancy. Each sample cup had an even amount of sample (30 ml) measured using a standardized jigger. The sample cups were served on a white 6 in X 8 in serving tray. A cup containing water at room temperature was provided for subjects to wash their palates in between samples. The panelists were also provided with unsalted crackers (Gillett and others 1984). The samples were covered with foil and served with a short straw to avoid visual differentiation.

The testing was carried out in individual testing booths in the Family and Food Science sensory testing facility, Room 105, at California State University, Fresno, which accommodates five subjects at a time. A total of 30 subjects were randomly selected. Each subject received written as well as oral instructions on the testing procedure along with a consent form (Appendix A). They were all informed that the samples may contain milk allergens. The subjects were then instructed to taste three samples and select the odd one out from each set with instructions provided on the testing sheet (Appendix B).

**Market Survey**

A market study was done to assess the preferred flavor, texture, and mouth feel for the beverage. Fifty surveys were completed at the following locations: California State University, Fresno campus; a Save Mart grocery store in Fresno, CA; a local convenience store (Save On Liquor, Fresno, CA); and the Student
Recreation Center at California State University, Fresno. All participants were all asked for their preference of flavors and beverage thickness (see Appendix C).

**Stabilization and Viscosity Studies**

The viscosity of various protein-based beverages available in the market (Muscle Milk, EAS’s Myoplex, Muscle Tech’s NOS, Naked Juice, Odwalla’s Superprotein, Isopure, Sunnyside Yogurt Smoothie, Bolthouse Farm’s Soy Protein Drink) were measured using a Brookfield viscometer using spindle number 5 at 30 revolutions per minute. Three readings were taken at intervals of 1 min each and the average of the three readings was taken.

Whey was blended with varied concentrations (0.5% and 1%) of the two stabilizers until the desired viscosity was obtained. Viscosities were measured using the Brookfield viscometer under the same conditions (Three readings with spindle no. 5 at 30 rpm for 1 min).

**Stabilizer’s Effect on Post Pasteurization Precipitation of Whey Protein**

In a study conducted by Ibanoglu in 2005 whey protein isolates retained higher thermal stability and did not denature at high temperatures when mixed with hydrocolloids. Thermal stability was even higher with pectin at acidic pH. This is attributed to increased hydrogen bonding (Ibanoglu 2005). Thus, whey with a sufficient amount of stabilizers would not denature and would also make a thicker full-bodied beverage by contributing to a better mouth feel (Moirano 1966). Moreover, hydrocolloids are and have been used in the beverage industry to create, blend, and even mask undesirable flavors (Glicksman and Farkas 1966).
Once the ideal concentration of stabilizer to be added was established, studies were done to determine their efficacy. A settling test was performed to check for amount of precipitation over time.

Three sample sets were prepared. One contained whey with 1% Freedom Gum X-PGA/LV Powder. The second sample contained whey with 1% TIC Pretested® Dairyblend 603-EP FF. A third sample, the standard, contained only whey. Each of these were distributed into five, 50mL centrifuge tubes that were pre-weighed and labeled. All tubes were placed in a test tube rack and placed in a water bath pre-heated to 72°C. The tubes were labeled. Once the temperature inside the tubes reached 72°C, the tubes were held for 10 min and then cooled down to room temperature. One tube of each sample was centrifuged and the precipitate was weighed at varying time intervals (0 h, 24 h, 48 h, 72 h, and 1 wk). The samples were centrifuged at 3,500 rpm for 10 min. The weights of precipitates over time were compared to determine whether the stabilizer was effective in preventing settling.

**Formulation**

Once a clear liquid was obtained from charcoal adsorption, fruit, flavor, sugar, stabilizer, and coloring agents were added. Each ingredient was tested in increments keeping the others constant. Visual and sensory observations were made subjectively to arrive at the suitable formulation. Further sensory evaluation in the form of a preference test or consumer acceptability test will be employed to test the beverage’s acceptability.

**Sensory Evaluation of the Final Formulation**

A simple acceptance test for the whey beverage was conducted using a 9-point hedonic rating scale. The objective was to determine overall acceptability of
the formulation. Scale ratings were converted to numerical scores: 9, like extremely; 8, like very much; 7, like moderately; 6, like slightly; 5, neither like nor dislike; 4, dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely. In order for the beverage to be considered “acceptable” by consumers, 75% of the total responses should be >6 on the established scale (Meilgaard and others 2007).

Subject Selection

One hundred untrained subjects were randomly selected from the Student Recreation Center, at California State University, Fresno to best estimate the target demographic. A Human Subjects Review Committee from the Food Science and Nutrition Department assessed the participants to be at “minimal risk.” The consent waiver forms (Appendix A) will be stored, on file in the Food Science and Nutrition Department office for 5 years.

Sample Preparation

The formulation was prepared as per protocol in the Material and Methods Section and includes the following ingredients: charcoal adsorbed cheese whey (obtained from the manufacture of mozzarella cheese from Dairy Processing, California State University, Fresno), stabilizer – Freedom Blend (obtained from TIC gums, USA), sugar, and fruit.

A single sample in a 2-oz cup was presented to each subject on a tray with a score sheet and a pencil. A cup of tepid water was offered to cleanse the palate before tasting (Meilgaard and others 2007).
Testing Procedure

The testing was held outside the Student Recreation Center at California State University, Fresno. A table is to be set up at the location allotted. The beverage is to be stored in coolers and the participants shall be served the beverage in 2-oz cups and instructed to mark their response on the hedonic score sheet provided (Appendix D). They were also asked to provide comments on what they liked or disliked in particular.
CHAPTER 4: RESULTS AND DISCUSSION

Whey Refining by Charcoal Adsorption

Activated carbon due to its intricate and well distributed porous structure and large surface area is a strong adsorption agent commonly used for water purification, decolorization, filtration, etc. The charcoal is treated with oxygen to increase its porosity and thereby making it “active.” The amount of adsorption is dependent on several factors such as temperature, pH, nature, concentration of adsorbate, and concentration of adsorbent (charcoal). In this study, the adsorbate is unknown and hence its nature and concentration cannot be determined. The pH is also kept constant at 5.6 (pH of whey). The temperature, concentration of charcoal, and time interval, however, can be subjected to change in order to determine the most efficient adsorption.

Effect of Temperature on Charcoal Adsorption

Charcoal adsorption was successfully attained at varying temperatures keeping concentration and time constant. It was observed that as temperature increased, there was a reduction in adsorption (Figure 2). The temperature at which maximum percentage clearance was observed (81.6%) was room temperature (25°C). At lower temperatures (5°C and 10°C), the adsorption curve lowered again. This could be indicative of either an optimum temperature being reached or that the reaction is endothermic or both. It may also be probable that the deterring compounds may be aromatic compounds.

Adsorption by activated carbon has been found to produce both endothermic (Namasivayam and Kavitha 2002) and equilibrium (Quadeer and Hanif 1994) reactions. In both these studies, the adsorbates were metal ions and
hence probably had similar characteristics of adsorption whereby adsorption increased with an increase in temperature. In the study of adsorption of phenolics, however, higher adsorption was observed at a lower temperature (24 °C) than at a higher temperature (60 °C) (Parajó and others 1996). The color and flavor compounds in whey are unknown thus making it improbable to chart an isotherm without knowing the adsorbate concentration. It was observed, however, that with adsorption of these unknown compounds, adsorption showed a reduced mean with increase in temperature.

Figure 2 – Effect of temperature on charcoal adsorption
Effect of Charcoal Concentration and Adsorption Time on Whey Refining

Adsorption of color compounds in whey at varying times and adsorbent concentrations at constant temperature (25°C) were achieved at all times and concentrations tested. Although the adsorbate in whey is unknown, the effect of time was found to be insignificant (P<0.05). In most cases, the concentration of adsorbate and adsorbent both, or their ratio thereof, has an impact on the level of adsorption (Parajó and others 1996). There is no specific trend to indicate an increase or decrease in adsorption with the level of adsorbate (whey compound) being added as it may be specific to the adsorbent. In the case of the cheesy odor and color of whey as well, concentration of charcoal showed significant difference in adsorption rates. A comparison of means showed that maximum absorbance was achieved at 1 g of charcoal per 100 ml of whey. Percentage color removal at this concentration was 76.4%. There was no significant interaction between factors (effects of time and concentration were independent of each other) as inferred from Tukey’s test on SPSS.

In a study conducted by Parajó and others and others in 1996 on adsorption of phenolic compounds from wood hydrolysates by activated carbon, different contact times were tested and equilibrium of adsorption was attained at the same level in each instance. They concluded that time was insignificant for adsorption (see Figure 3).

Sensory Evaluation to Determine Efficacy of Charcoal Adsorption

The Triangle Test was analyzed using Table T8 Triangle Test for Difference: Critical Number (Minimum) of Correct Answers (Meilgaard and others 2007). The respondents of 60 sample sets were recorded, of which 55%
responded correctly thus indicating, as per Table T8 mentioned above, that there is a significant difference in the two samples at $\alpha = 0.05$.

### Figure 3 – Effect of charcoal concentration on color removal

![Graph showing the effect of charcoal concentration on color removal.](image)

**Market Survey**

A market survey of 50 subjects was conducted and was indicative of a strong preference for mixed fruit flavors. Forty-four percent of the subjects expressed a preference for strawberry amongst other fruit flavors, and 58% preferred a thick, smoothie-like beverage as opposed to a drink with thin, juice-like consistency. Based on this survey, the blend devised for this study was a thick smoothie with strawberry and banana flavor.
Stabilization and Viscosity Studies

Weights of precipitates over time for standard (whey), whey with Freedom blend stabilizer (1:100 w/v), and whey with Dairyblend stabilizer (1:100 w/v) were analyzed and plotted on a graph, using PSI plot (see Figure 4). Solution stability maintained better with both stabilizers as compared to whey alone. The whey with Freedom blend had less precipitation compared to both the standard and the Dairyblend over all time intervals tested for as exemplified in Figure 5. The Dairyblend is comprised of pectin, guar gum, propylene glycol alginate, and dextrin, whereas the Freedom blend contains xanthan gum, guar gum, Maltodextrin, and propylene glycol alginate. Xanthan gum has better stabilization properties due to the weak gel formation that occurs due to the dispersion of xanthan polysaccharides in the continuous phase. Pectin, on the other hand, at lower concentrations is unable to create a network of colloidal particles large enough to prevent phase separation. At higher concentrations, pectin disrupts the rheological properties of the mixture and affects consumer acceptability of the beverage (Parker and others 1995).

It has been observed in other studies, such as one conducted in 2003 by Paraskevopoulou and others, while testing various polysaccharides to study their stabilization effects in a cheese whey, kefir drink, that Xanthan gum was far more effective than pectin even when higher amounts of pectin were added.

Formulation

Based on the aforementioned research, the beverage formulated had a composition of 40 g strawberry, 10 g banana, 0.2 g stabilizer, and 25 g sugar per 100 ml of whey. The pH of the final beverage was 4.65 and sugar was at 17°Brix.
Figure 4 – Comparative analysis of the effects stabilizers on the precipitation of proteins in whey

Figure 5 – Comparison of precipitate with whey blended with stabilizer (right), and plain whey (left), centrifuged after storage for 72 h.
Sensory Evaluation for Finished Beverage

On the hedonic scale from 1 to 9, 95% of the 100 subjects tested rated the beverage as 7 and above. The formulation was successful in presenting itself to be a palatable beverage by foregoing over 75% acceptance level. Some variability exists in the amiability of the beverage in terms of viscosity and mouth feel. This is based on the consumer’s individual opinions. Most consumers as per Food Trend predictions for 2008-09 prefer a thicker “smoothie-like” beverage. There were no comments stating any hint of dairy or cheese-like odor. Some of the comments left by the subjects included, “Was great!! I would definitely buy it!” “Great Tasting!” “Flavorful and very inviting!” “Delicious,” “Tastes just like Jamba Juice!!”
CHAPTER 5: CONCLUSION

The overall objective of this study was to develop a novel technique for utilizing whey in a beverage.

The undesirable color and cheesy odor in whey was significantly reduced by the process of charcoal adsorption. A charcoal concentration of 0.5% (w/v) at 25°C removed up to 81.63% of color. The color removal was instantaneous with no time effect. The most ideal stabilizer tested was Dairyblend™, which reduced post pasteurization protein precipitation and also increased product viscosity at a concentration of 0.25% (w/v). The Consumer Acceptance Test showed the formulated beverage to be highly acceptable. The beverage was ranked ≥ 7 on a 9-point hedonic scale by 95% of the subjects tested.

Future Work

This research was put together to formulate a nutrient beverage utilizing the materials at hand. In order for it to be replete and marketed effectively, modifications to improve the overall appeal and palatability needs to be assessed. This can be accomplished by adjusting the taste, flavor profile, body, mouth feel, Brix, etc. Furthermore, studies to test for shelf stability and preservation tactics would be necessary. Nutrient assessment as per labeling laws also need to be conducted in order to achieve regulatory consent.
REFERENCES
REFERENCES


Huffman, LM. 1996. Processing whey protein for use as a food ingredient. Food Tech. 50:49-52


differences in leucine kinetics and nitrogen balance in endurance athletes. J.
Appl. Physiol. 75:2134-41.

of thorium adsorption on activated charcoal from aqueous solutions. Journal
of Islamic Academy of Sciences 7(1):56-60.

Roeder, G. 1939. Improved process for manufacturing fermented beverages. J.

Rosenbloom, CA. 2000. Sports nutrition, a guide for the professional working


Smithers, GW, Ballard, FJ, Copeland, AD, de Silva, KJ, Dionysius, DA, Francis,
GL, Goddard, C, Grieve, PA, McIntosh, GH, Mitchell, IR, Pearce, RJ,
Regester, GO. 1996. New opportunities from the isolation and utilization of

Tarnopolsky, MA, Atkinson, SA, MacDougall, JD, Chesley, A, Phillips, S,
Schwarz, HP. 1992. Evaluation of protein requirements for trained strength

Tipton, KD, Wolfe, RR. 2004. Protein and amino acids for athletes. Journal of


Yoo, BW, Mattick, JF. 1969. Utilization of acid and sweet cheese whey in wine
APPENDIX A: CONSENT WAIVER FORM
CONSENT FORM

CALIFORNIA STATE UNIVERSITY, FRESNO

DEPARTMENT OF FOOD SCIENCE AND NUTRITION

CONSENT TO PARTICIPATE IN THE FOLLOWING STUDY

I, (print name). ____________________________ hereby willingly and voluntarily consent to participate as a subject in the research project entitled

“Development of a Beverage from Whey” conducted by Dr Dennis Ferris, and Akshay Aswani at California State University, Fresno. The Food Science and Nutrition department committee at California State University, Fresno has reviewed and approved the procedures for this research, which is determined to be of minimal risk to participants.

I understand the procedures for participating in the project are as follows:

I will taste a sample of the whey and record my results on an accompanying score sheet.

To the best of my knowledge, I have no known allergies to whey, milk or dairy based products.

I understand that the results of the study will be kept on file at California State University Fresno in the Food Science dept and will be kept confidential.

I understand that I may quit or decline to participate in the study at any time.

I fully understand the terms of my consent and agree to participate in the study.

Signed ____________________________________________________________________________

Date: ____________________________________________________________________________
APPENDIX B: TRIANGLE TEST
Triangle Test

Taster no. _____ Name__________________________ Date__________

Type of sample: Whey

Instructions:
• Rinse with Water before starting
• Taste Samples on the tray from left to right
• Two samples are identical; one is different. Determine the odd/different sample. Circle the code of the odd sample. If no difference is apparent, you must guess. After completing one set, rinse palate with cracker and water that has been provided before starting on the next set.

Trial 1: 786  316  511

Trial 2: 441  689  352
Market Research Survey:

Akshay Aswani

M.S. Food Science and Nutrition

Age Group:
1. 15-20
2. 20-25
3. 25-35
4. 35 and over

Sex:
1. Male
2. Female

Physical Activity:
1. Athlete
2. Recreational Weight lifting
3. Aerobics/ Cardiovascular
4. Cross-Training
5. Couch Surfing

Time Spent on Physical Activity:
1. Once a week
2. 2-3 times a week
3. 4 or more times a week
4. Constantly shredding
5. Annually

Do you consume Ready to Drink Protein Beverages?
1. Yes
2. No
3. Never heard of them
4. What is protein?

How often would you say you consume protein RTDs?
1. Just about everyday
2. Once a week
3. 2-3 times a week
4. Rarely
5. Never
How much would you pay for a RTD protein Drink?
1. $1-$2
2. $2-$3
3. $3-$4
4. $4-$5

What flavor/s would you like to see in a protein drink?
1. Chocolate
2. Banana
3. Strawberry
4. Vanilla
5. Grape
6. Any other _____________________

Do you prefer…
1. Thin juice like consistency?
2. Thick (Smoothie, Jamba Juice)
3. Yogurt like
4. Gooey like melted chocolate

What do you like/not like about the currently available beverage
APPENDIX D: CONSUMER ACCEPTANCE TEST
Consumer Acceptance Test

Name ________________________   Date ______________________

Sample – Whey Beverage

- Please rinse palate with water provided
- Evaluate the sample provided for appearance, taste, aroma and mouth feel
- Indicate your overall opinion by checking one box

Dislike Extremely
Dislike Very Much
Dislike Moderately
Dislike Slightly
Neither Like nor Dislike
Like Slightly
Like Moderately
Like Very Much
Like Extremely

Comments:
California State University, Fresno

Non-Exclusive Distribution License
(to make your thesis available electronically via the library’s eCollections database)

By submitting this license, you (the author or copyright holder) grant to CSU, Fresno Digital Scholar the non-exclusive right to reproduce, translate (as defined in the next paragraph), and/or distribute your submission (including the abstract) worldwide in print and electronic format and in any medium, including but not limited to audio or video.

You agree that CSU, Fresno may, without changing the content, translate the submission to any medium or format for the purpose of preservation.

You also agree that the submission is your original work, and that you have the right to grant the rights contained in this license. You also represent that your submission does not, to the best of your knowledge, infringe upon anyone’s copyright.

If the submission reproduces material for which you do not hold copyright and that would not be considered fair use outside the copyright law, you represent that you have obtained the unrestricted permission of the copyright owner to grant CSU, Fresno the rights required by this license, and that such third-party material is clearly identified and acknowledged within the text or content of the submission.

If the submission is based upon work that has been sponsored or supported by an agency or organization other than California State University, Fresno, you represent that you have fulfilled any right of review or other obligations required by such contract or agreement.

California State University, Fresno will clearly identify your name as the author or owner of the submission and will not make any alteration, other than as allowed by this license, to your submission. **By typing your name and date in the fields below, you indicate your agreement to the terms of this distribution license.**

<table>
<thead>
<tr>
<th>Akshay Aswani</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type full name as it appears on submission</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>November 20th 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
</tr>
</tbody>
</table>