

ABSTRACT

SHARP, MATTHEW ALAN ROBINSON. Development of a Shelf-stable Caloric Dense Protein Bar Using a High Fat System (Under the Direction of Dr. Gabriel Keith Harris).

In 2019 protein bars became a 9.2 billion-dollar market-making up 19% of the total bar market. The Protein bars on the market range from 6% to 40% of the total mass of the protein bars currently on the market, and the most common flavor is chocolate. For thousands of years, man has produced highly nutritious, portable foods in the form of dried meats. In the late 1800s, granola was invented. Beginning in the 1960s and 1970s, granola bars and other bar products were created as tasty snacks to improve athletic performance or to provide additional protein. Since Napoleon's time, it has been widely recognized that an army "travels on its stomach". The need for portable food for the military resulted in canning technology. Cans were effective but heavy. Mans' excursion into space introduced the first protein bar and the importance of weight limitations while traveling. MREs, or meals ready to eat, created in 1975 made use of plastic pouches and cardboard containers to provide nutrition without excess weight. While they were an improvement over previous rations, MREs were often "field stripped" to eliminate the excess bulk and weight of packaging, as well as to get rid of unwanted food items. Given the need to expend over 6,000 kcal per day during some forms of military operations, a new, highly portable, highly palatable, energy-dense food ration was needed. The primary objective of this project was to create a high-protein, high-calorie bar with extended shelf life. One of the primary challenges that had to be addressed was the bar hardening resulting from protein aggregation. Bar hardening was addressed by enrobing dairy proteins in specific types of solid fat to prevent bar hardening and achieve other desired outcomes. When encapsulating whey protein into

a matrix that is 50% whey and 50% in specific types of lipids, it introduces a calorically dense product that addresses protein self-association. FDA has no specific regulations regarding nutrient-dense claims nor adding caffeine to food products. The result of this project was the creation of the Soldier Boost bar, which later came to be known as the Soldier On bar. Soldier on bar contains 19 g saturated fat, 19 g protein, and 19 g of carbohydrates in a 65 g product that achieves a melt-point above 55°C. Soldier On bar withstands temperatures of 55°C while maintaining structural integrity. After a 30 day self-life study, statistics indicated that storage temperature does not affect the color of the protein bar, but the length of time is the primary driver of color change. Vitamin C introduced to the protein bars indicated that at 22°C, Soldier On bar would have the highest retention at lower temperatures unless Vitamin C is inculcated prior to production. Residual fat can separate in simple protein-lipid matrixes and decrease the melt-point temperature below the desired 55°C. The enrobing technology required to create the bars resulted in a US and International patent application. This project was a collaboration among the Defense Logistics Agency of the Department of Defense, the primary sponsor, along with SMRC (Systems and Materials Research Corporation), and NC State University.

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Development of a Shelf-stable Caloric Dense Protein Bar Using a High Fat System

by
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DEDICATION

This is dedicated to all my family, friends, and people who have helped and supported me over the years as I have gotten my higher education degrees. A special thank you, Mom, for all you do for me, and I am sure you will do for me in the years to come.

BIOGRAPHY

Matthew Alan Robinson Sharp was born in Washington State. Matthew grew up in Issaquah, Washington as an only child. In high school, he competed in wrestling, cross country, and soccer while actively playing soccer with a competitive year-round team. Matthew, in his spare time, enjoyed snowboarding, playing pick-up games of ice hockey, and working in different food industry jobs. When not in school or participating in his long list of activities, he spent time with his closest friends, Matthew, Jeffery, and Joseph.

After high school, he earned an Associate of Applied Science at the Art Institute of Culinary Arts in Charlotte, NC, in 2009. Matthew received an Associate of Arts degree in 2013 in business administration at Wake Technical Community College. In 2017, Matthew obtained a Bachelor's in food science from North Carolina State University.

In his free time, he plays video games, watches movies, and is always searching for local area restaurants that offer preeminently awesome food and drink. While Matthew has been in school, he has been motivated by his closest friends, grandparents, and his mother, who have provided moral support, encouragement, and at times jokes about taking too long to finish school. Matthew's other hobbies include (but are not limited to): building Lego® sets, camping, hiking, golfing, snowboarding, playing and watching soccer, watching hockey and football games, cooking great dishes for friends and family, and reading science fiction books.

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Chapter 1: Review of The Literature

The History of Protein Bars

Protein bars are a relatively new product of the evolution of the food industry, as represented in Table 1 History Leading up to Current Product Market. In comparison, dried meats or other dried, high protein foods can be traced back over 5,000 years to Egypt and have been found in tombs dated 3,200 BC. This form of dried protein food was used widely across Africa, the cold-weather climates in Europe, and South America (Ngapo et al 2021). Jerky is not technically considered a protein bar in the current definition, but the concept is the same. Jerky can be produced from beef, chicken, duck, pork, salmon, turkey, venison, or other foods that contain high protein and fat, such as tofu (Sherow 2015). Often, other ingredients are mixed in with protein-rich ingredients to provide flavor or other desirable properties. One such item is pemmican, which is prepared from dried meat strips, tallow, and dried berries. This traditional dish was created to help provide a portable ration while because of its light weight and the fact that it served as a great source of protein, fat, and other nutrients. In this way, it was similar to today's nutrient-dense protein bars (Brendza ; Augustyn et al; SousvideGuy ; Lum; Bull and Cleaver).

Granola

Granola was first invented in 1863 by James Caleb Jackson at the Jackson sanitarium. It consisted of graham flour that was rolled into a flat sheet, baked, crushed, and baked for a second time. This was consumed with milk as a cereal, providing the paradigm for what people think of as granola cereal today (Vásquez 2001). In the 1960s, granola was revitalized with the addition of dried fruits and nuts to the recipe,

and it gained renewed popularity (Heartland 2021). The rights and patents were sold numerous times between 1964 and 1972, when the Quaker® company acquired them and created what we today call the granola bar (Hochman 2009).

Space Travel

The first iteration of a protein bar that was processed beyond just seasoning and smoking meat was developed by NASA in the 1960s for astronauts under the name "Space Food Sticks." It was different compared to the other rations since it was not a freeze-dried product like most of the other space foods. The company that made them created a range of flavors such as orange, peanut butter, and mint. Unlike the popular orange-flavored Tang® beverage, "Space Food Sticks" did not successfully transition to the public sector. Experts speculate that the product failed because it was a meal replacement or was not appealing. While in 2020, meal replacement bars are considered a positive and even necessary category of food for busy consumers on the go, this was not true during the 1960s when the focus was on sitting down to family meals (Bourland 1993; Perchonok 2002; Bourland 1993).

Snack Bars and Nutrition Bars

Although NASA's original Space Food Sticks were not successful, snack bars did gain in popularity during a similar time frame, creating a new market for snack and nutrition bars. Snack bars were typically made with granola "glued" together with a sugar syrup. Dried fruit, chocolate, or nuts were often incorporated into the bars to create a multi-component, portable snack. Nutrition bars were also created at a similar time as a fortified nutrition product, targeting average consumers rather than being made specifically for athletes. One example of these nutrition bars is the Nutrigrain®

cereal bar. A secondary category of nutrition bars were breakfast replacements bars designed for “on-the-go” individuals, similar to Pop-Tarts®. These products were developed using wheat flour or milled oats and sweetened, fruit-based fillings. Table 2 shows example products from each of these categories **Table 3** Common Bar Market Examples with Macronutrients information of each of the different piece of the bar market, while **Table 5** and **Table 6** matches activity levels with bars that best fits the activity and Figure 1 shows the breakdown of the market by percentage.

Performance Bars

In 1986, under the name energy bars, the PowerBar® was formulated and released to the public. Later, this would fall under the performance bar category. This category of bars has been used by athletes to promote recovery, increase energy during exercise, or as a post-workout snack. These performance bars have been created using sugars, protein, and stabilizers that were mixed then extruded to create a reliable single texture product. This allowed for a rapid increase in energy or recovery after a workout to allow the athlete to feel energized (PowerBar 2015). These performance bars gained popularity around the year 2000 as consumers diversified their diets and started to move away from consuming traditional breakfasts.

Weight Loss Bars

A recent addition to the bar category is weight loss bars, used to help lose weight while providing the nutritional needs of a healthy meal. Weight loss bars were created as a lower-calorie meal replacement of nutrition bars. The same companies that released them also introduced meal replacement shakes to appeal to a broader

customer base (Rothacker 2004; Tieken et al 2007). Each bar type was created to fit a need in the market over the years.

Protein Bars

The newest development is a cross between the nutrition bars and performance bars. These are high protein bar used by ordinary people who work out but are not high-performance athletes. People now have other hybrids of weight loss bars and performance bars to help those who want to increase muscle mass and work out but still maintaining one's diet. A similar issue of providing protein and nutritional content to soldiers has become an enormous concern over the past 20 years as soldiers have changed how they want to eat.

Military Rations

The United States military has around 2.1 million troops across active military, reservist, and National Guard units. With a military this size, a significant concern is how to properly feed and maintain the troops' readiness. Feeding a large number of men and women in the armed forces required meal facilities at all military installations, but similar to Napoleon trying to conquer Europe, troops on the march need food as well. Napoleon created a contest to find a way to feed troops. The result of this contest was the invention of glass canning in 1810 by Appert (Ortved 2020). These glass-canned rations were first used to support Napoleon's troops, but quickly became a standard means of preserving food, not only for the military, but for the public in general. Napoleon's contest created the first version of a premade meals for soldiers out in the trenches that were not simply dried foods or needed to be reheated in a full kitchen, mess hall, or in some branches chow hall. In the United States, during the

Revolutionary War, military soldiers consumed garrison rations that had salted fish or meat with bread and vegetables or a spirit ration that contained up to four ounces of rum or whisky (Koehler 1958). The alcohol was later replaced with coffee in 1846. A Condensed timeline is expressed in **Table 4** History of Military Meals.

US Military Rations in World War I

During World War I, the Emergency or Iron ration, Trench ration, and the Reserve rations were used. These came in tin or metal cans with some meat, bread or biscuit, ground coffee, and cigarettes. The Iron ration was used between 1907 and 1922, but fell out of fashion since they caused meal fatigue due to their lack of variety. In 1914, Trench rations were produced but only lasted until 1918. In contrast, the Reserve ration was adopted in 1917 and used until 1937. As a result of World War I, the military created five different rations meals known as A, B, C, D, and K Rations. Each of these had been formulated for different types of environments. A Rations are fresh, so refrigeration is needed; this type of food was only found at bases. B Rations only needed a field kitchen or a place to eat the food, but no refrigeration was necessary. The C Ration was reformulated and renamed "Meal Combat Individual" (MCI). Later, water-containing versions were referred to as "Wet MCI". In contrast, K Rations were used for short assaults and typically provided to paratroopers. Both the D and K Rations are considered obsolete and are no longer in use (Cleaves 1945; Patterson 1945; Longino 1946).

Individual Meals

Between the use of MCI and the introduction of the Wet MCI, the military went from one meal type to twelve different meals to reduce fatigue. As the MCI was a single

entrée meal and the Wet MCI added the eleven other combinations, this made for a total of twelve different meals that soldiers could consume. The MCI, however, had a few flaws in its design (Koehler). One of the greatest issues with the meals was that they were nutritionally inadequate for the soldiers and were extremely heavy because they were packaged as cans inside cans. In 1975, the Meal Ready to Eat (MRE) was invented. Instead of cans, MREs were contained in light, flexible plastic pouches and cardboard packaging. Testing MRE's took three years for mass production, shelf-life tests, and phasing out the wet MCI. Soldiers did not see the first MRE until 1981 (Feagans et al 2010). The MRE present in the early 1980's still needed improvement as the soldiers only consumed about 60% of the calories, which led to the creation of the MRE XVIII that started production in 1988 and increased the meal selections from nine to twelve. The menu continued to expand to twenty-four different meals by 2003. While the meals are revisited and changed every two to three years, the total number of meals has stayed the same.

The military has changed the meals and increased the variability of meal options for the soldiers. Some of these meal options have not always had positive reviews from the soldiers; the most disliked MRE was the vegetarian omelet produced between 2005 and 2008. Soldiers promptly nicknamed the vegetarian omelet the "vomlet," MRE (Hawver 2006; Stilwell 2015; Lunn 2019). The US Army Natick soldier systems center fifteen years later still receives complaints about the "vomlet" from military personnel and civilians alike. With both successful and failed meals in the past thirty years, the military is now looking for extra supplemental meals to expand the current mixture of MRE's (Gonzales 2005). Soldiers cannot eat four or more MRE's in a day while still keeping

mobile and combat-ready. Eating four or more MRE's might seem like an easy task, but this is analogous to eating four large family dinners in a single day. To help achieve the 6,000+ kcal a day requirement, they have to find other methods of increasing ration intake. Today's 18 to 30 year old soldiers do not think about meals and food in the same manner as soldiers in the mid 1990s when the current MRE's had been mainstreamed and accepted not only by the military but also by civilians as acceptable meal options (Feagans et al 2010). Instead, today's soldiers are looking for fast, easy, and ready-made meals that can be eaten while moving, not sitting on the ground waiting for the main course to be heated up or cooked.

The Problem of Field Stripping

The United States military have continuing issues with soldiers "field stripping" their MRE. The concept of field stripping involves removing all the extra packaging, such as the cardboard box holding the main course, removing extra plastic wrap, trading or throwing out items they do not want to eat, and then placing them in their pouch or pockets. Some of the reasons they do not keep items might not be a result of not liking the item, but if it takes long to prepare or cannot be eaten while marching, then they toss it out. Paper products are kept to start fires to either keep them warm or to help heat the food later (Dominguez et al 2018). This can introduce the issue of them not consuming enough calories in their diet due to field stripping. Soldiers need upwards of 6000 kcal or more each day in some extreme weather conditions (Beals et al 2019). Field stripping of rations is not unique to the U.S. military. It has been reported to occur with military troops, such as those in Norway, even when cold weather places additional calorie demands on troops just to stay warm.

The change has caused the military to look at what today's soldiers might choose to eat instead while on the go. A shelf-stable calorically dense protein bar could help address this need. This led to the creation of the first few attempts to fulfill the military's needs, such as the paratrooper bar. The soldiers, although this was an attempt to fix the problem they noticed more of the bars in the trash and less being eaten. Not only had the new bars not been eaten but it was placed into the MRE's meal kits not placed as an added ration. The need for a highly portable, enjoyable ration separate from MREs resulted in a call from the military for the development of a new, shelf-stable, calorically dense protein bar.

Nutrient Claim Regulations

Regulations currently expressed in 2020 on the food industry are vast and cover most of the food industry, especially when it comes to claims on products. While many claims can be used, like "contains 100 Calories" or "Fat-Free" are addressed in the Code of Federal Regulations title 21 (21 CFR). The regulations in the United States currently focus on claims that indicate low, no, or light amounts of ingredients and do not directly address the high-level claims like "nutrient-dense" or "high fiber." Claims such as nutrient-dense, high protein, and high fat are not defined by federal regulations. Low, no, and light claims come with the maximum allowed of the product. One example of this is fat-free milk still has fat but it cannot exceed 0.5% percent of the milk. Caffeine, on the other hand, is not currently regulated when added to food products like protein bars or pizza, but it is regulated in soda and beverages.

Caffeine Additives

Aside from coffee, the military has experimented with addition of gum and other products such as caffeine in order to keep soldiers awake and alert. According to 21 CFR 182.1180, caffeine is declared generally recognized as safe or GRAS in soda beverages at 0.02% percent or 200 parts per million (ppm) (21 CFR). Energy drinks, however, are not classified as soda but food supplements, and they contain amounts above 200 ppm. Additionally, the only current regulation on caffeine is focused on the potentially lethal amount in a food system. The estimated acceptable level is the same amount as a cup of coffee. The average amount in coffee is 80mg of caffeine (Mattia and Director ; FDA 2018a; FDA 2018b). Protein bars may be created with or without caffeine in order to provide options for soldiers in terms of caffeine intake.

Supplement Labels

While food products are regulated by the FDA (CFR 21), supplements are not as strictly regulated as food products. This leaves a gray area that allows people to create food and beverages under the supplement label that could be sold in places like GMC® but not go through the same safety standards that major brands like a Cliff® bar or Nutrigrain® cereal bars go through to be sold commercially. When vitamins and caffeine are added to a protein bar, they can then label it a supplement. For the most part, the military focuses on the provision of foods, rather than supplements. Depending on the formulation, proteins bars or drinks could be marketed as either foods or as supplements.

Protein Bar Market

According to a study conducted by Mintel in 2007, (Mintel 2007), the protein bar market has increased by 18.5% between 2001 and 2006. Between 2006 and 2019, the protein bar market showed a total combined income of 9.22-billion-dollars, with a 17% increase between 2013 and 2019. The projected single-year income for 2024 may be as high as 9.19 billion dollars, showing a dramatic increase in demand compared with the previous decade. Consumers are increasingly interested in healthier options, eating on the go, and becoming more time-efficient. The economic impact of 9.22-billion dollars spent in the industry is very important. Understanding what the consumer considers important as trends will spur sales to either continue as projected, decrease or surpass the expectation. Protein is considered the most important for the developers since 52% of consumers look at the amount of protein in the bars while considering which brand to purchase, followed by the amount of sugar at 43%, and price being a concern with 34% of consumers. Snack bars are seizing a coveted spot in the market since 19.7% of consumers are looking for protein bars or other performance bars, while other nutrition bars are sitting at 19.1% and taking the most significant majority of 46.5% of the consumers looking for snack bars according to a 2017 report (Mintel 2017).

The largest sale holders of all forms of bars come from supermarkets at 14% and the "other" being supercenters, club stores, natural food stores, and online retailers with 19%. The market shares of snack nutrition and performance bars by companies are General Mills® hold 22% of the market, Kellogg® holds 14%, Clif bar® holds 12%, and "other" comprises small brands making up 20% of the total market, while Kind®, Pepsi co®, Alkins®, Quest®, and Private labels are making up a combined 32% market with

none larger than 9%. This can also be seen in Figure 2 Shares of Sales by company over 52 weeks in 2019. Most protein bars use whey protein or soy protein, sucrose or high fructose corn syrup, stabilizers, and a minimal amount of fat (Mintel 2007; Mintel 2009; Mintel 2010; Mintel 2012; Mintel 2014; Mintel 2016; Mintel 2017; Mintel 2018; Mintel 2020).

Protein Bar Formulations

The traditional composition of protein bars is about 10-30% protein, 40-70% sucrose in some form, 10% fat or lipids for texture, and up to 10- 40% stabilizers, carbohydrates, and other fillers used to create a more appetizing or nutritious product. One such example of this is the Powerbar® Chocolate flavor that contains 70% sucrose, 3.1% fat, 14% protein, and 13% vitamins and minerals (Mintel 2007). This product mostly consists of sucrose and sucrose enhancers, oats, soy protein isolate, cocoa powder, rice flour, and less than 2% vitamins. To make it sweet, they use a high amount of sucrose mixtures, a little bit of protein to make it qualify as a protein bar, and enough fat to help with mouthfeel, and so the bar can serve as a carrier for fat-soluble vitamins. The reason behind the massive amount of sucrose solutions is to provide quick carbohydrate energy, but this creates potential issues with crystallization and browning; thus, the manufacturers tend to make dark-colored bar products hide most of the undesirable coloring caused by the browning reactions.

Ingredients

Ingredients: Sugars and Caramelization

Protein bars have a substantial issue with a reaction called bar hardening. This is a result of two different reactions occurring. The first interaction that is of consequence

is that protein tends to self-associate to become a very tough hard object. The second obstacle has to do with sugars and creating too much caramelization as a result of the protein self-associating, allowing the sugar crystals to also self-associate and then caramelizing. The caramelization by itself is not a concern, but this reaction creates the glassy hard candy texture to the outer area of some protein bars (Keefer et al 2020). Protein bars will have a noticeable change in hardness after the first two to three months but become too hard to eat somewhere between four and six months from the production date (Taillie, 2006). This might seem like an acceptable timetable, but the regular industry needs protein bars to be shelf-stable for at least one year (Imtiaz et al 2012). All of this bar hardening can be attributed to the intermolecular disulfide bonds, the non-covalent interactions (Zhou et al 2008a; Zhou et al 2008b), and protein polymerization (Tran, 2009) with an addition of other factors like moisture migration in high sugar protein bars.

Ingredients: Whey Protein

High amounts of protein in the bars are part of the goal in the nutrient-dense product. Protein can come from two different categories animals or plants. The most common types of protein used in foods are whey protein isolate (WPI) (Adamson 2011) and soy protein isolate (Hogan et al 2012).

Ingredients: Cocoa Butter

When looking at lipid structures and shelf stability, choosing the saturated fat of cocoa butter because of its stable structure and its crystalline network could prevent the migration of partials like protein over time. If one takes the generic base of chocolate or cocoa butter as the main structure of the protein bar, then temperature resistance to

heat needs to be considered. One study indicated that microstructure, mechanical properties, and crystallization could be changed with either 1,2,3-tristearoyl-glycerol or 1,2,3-trilinoleyl-glycerol. These will transform the stable crystal structure from β_2 or formation 6 to a stable β_1 or formation 5 form. By changing the triacylglycerol profile of the cocoa butter, it will change the structure and either slow down the crystallizing process and or slow down the melting process once the product has solidified (Campos et al 2010).

Ingredients: Melt Point

While both β_1 and β_2 are stable and acceptable forms of cocoa butter, β_2 has a melting range of 32-34°C and β_1 has a melting range of 34-36°C. Other formations to note are Gamma (γ), or structure 1, which has a melting point between 16-18°C. γ' structure 2 has a melting range of 22-24°C, α or structure 3 has a melting range of 24-26°C. α' or structure 4 has a melting point of 26-28°C. With the change in melt point becoming larger between the ending of α' at 28°C and the beginning of β at 32°C is 4 °C spacing while the other forms do not have this gap between crystal formations making β far superior in structure than the other formations as shown in **Table 7** Structure and Stability Ranking.

Structure 1 is the least stable of all the formations, while structure 6 is the most stable. (Beckett and Royal Society of Chemistry (Great Britain) 2018) This basic formation is determined by the level of detail needed. Most books and literature only cover the four significant forms γ , α β' and β (Vaeck 1960) with melting ranges of 17°C, 21-24°C, 27-29°C, and 34-35°C, respectively (Minifie 1999). The first four structures are seen as random transformations directly from melted cocoa butter, while phases 5 and

6 are believed to occur based on the "memory" or seeding effects of cocoa butter crystals added to the molten cocoa (Marangoni 2003; Schenk 2004; Fernandes et al 2013).

Ingredients: Crystal Structure Characteristics

Other characteristics relating to crystal formation are based on the type of chocolate product considered from straight cocoa butter, dark chocolate, or going as far as milk chocolate, as each product added new ingredients that modify how the crystal structure behaves and what kind of structures that can be achieved. As lecithin will give the chocolate better flow properties and decrease the polymorphic transition time while keeping the proper viscosity when used under 1 percent of the total product, typical industrial markets use close to 0.4 percent of lecithin in the final product (Rigolle et al 2015). When working with milk chocolate products, the milk powder's processing method combined with an emulsifier like lecithin will determine the final chocolate products' yield stress. Roller-dried milk powder had a higher Casson yield stress than spray-dried milk powder when added to cocoa butter with values $16.04 \pm .21$ and $14.15 \pm .08$ τ_{CA} (Pa), respectively (Ačkar et al 2015). Additional ingredients can affect the final product. The coating process addresses the phospholipid layers, changing its interactions with lecithin and making the product either more or less hydrophilic, causing the industry to increase or decrease the amount of lecithin needed for a stable chocolate bar. (Kindle et al 2018) The melting point of cocoa butter and chocolate can be a significant variable, especially in places like Afghanistan or other countries with sweltering weather.

Ingredients: Cocoa Butter Equivalents

To help combat this, companies use mixtures of Cocoa butter and other cocoa butter equivalent lipids (CBE), cocoa butter substitute (CBS), and cocoa butter replacers (CBR). CBE are fats that are compatible with cocoa butter, but some are more compatible than others based on the triacylglycerol composition. (Jahurul et al 2013) When adding other lipids to cocoa butter can affect the final crystal structure and lead to the formation of a chocolate defect called blooms. Blooms occur due to less stable crystal formations (γ , α , and β'). The improper formations might occur due to inadequate temperature and mixing procedures at the production facility or because of repeated heating and cooling during storage. (Bricknell 1998) One CBR used instead of cocoa butter is palm kernel oil, and in most situations, it is used for coating or enrobing chocolate products, and it is used in less than 5% of the total amount. (Biswas et al 2018) Allied fats is a term not commonly used but assuming 5% is placed into the total product it can be hard to source and contain symmetrical triglycerides to be equivalent to Cocoa butter (Minifie 1999). Examples of this can be found in **Table 8** Cocoa Butter Alternatives. This can occur because of its large difference in triacylglycerol composition.

Ingredients: Palm Kernel Oil

Another issue is that PKO's natural crystal formation is that β' and not the β (5 or 6) that is the ideal formulations in cocoa butter. Although CBR's and CBS's can be used in small quantities with little to no adverse effects in ideal circumstances, they can also be seen as incompatible fat in large quantities, creating unwanted blooming on the final product (Schenk 2004; Lonchamp 2004). Another side effect of using other CBS's and

CBR's in a food product is that the wrong proportion can result in an undesirable waxy texture or waxy mouthfeel. Although extenders and substations of cocoa butter can have negative aspects, it often has positive effects such as increased melting points when used in the right amount (Biswas et al 2017).

Palm kernel oil (PKO) is filled with medium-chain fatty acids, having a large amount of Lauric acid, making it a cocoa butter substitute. PKO is generally found in fat-rich products like ice cream and used in foods as a physical stabilizer (Chai et al 2018). PKO has an extensive range of melt points based on its processing method. This range can be anywhere between 32°C and 80°C. Increased use of palm oils has caused some backlash because some of the processing methods create a large number of trans fatty acids that have been connected to health problems (Fang et al 2010). When added to cocoa butter, this can create an increased melt point of the final product. This is often shown in confections that should be a melted puddle of chocolate at temperatures above 34°C, like hot summer days in North Carolina or Afghanistan (Biswas et al 2016).

Ingredients: Coconut Oil

Coconut oil is used as a cocoa butter substitute because it has a β crystal formation. Coconut oil can be solid or semisolid at room temperature, providing the ability to form similar products as chocolate but with a lower melt point. When coupled with natural flavors or chocolate powder, it can then mimic chocolate in products but does not create the characteristic snap associated with properly set chocolate. This is an advantage over cocoa butter because it does not require the same requirements cocoa butter does when used in food products. By using coconut oil, in place of cocoa

butter will prevent chocolate bloom on the outside of products and create a soft shell that provides a more pleasing mouthfeel in some products.

Analytical Methods

Analytical Methods: Hydrophobicity Methods

While hydrophobicity methods have been around for at least two decades, very few methods for determining hydrophobicity on dry solid foods are available. Most of the methods are for liquid foods. One method to determine hydrophobicity is to do hydrophobic gel chromatography with butyl Sepharose 4b based on the method of Hjerten et al (Hjertén et al 1974) that allows the person to determine the hydrophobicity of the product based on the speed at which it travels in the column. This is an acceptable method for protein powders in solution. It does not work for dry powders or other foods. Other methods used are interfacial and surface tensions of a droplet of the solution with only 0.02% protein and base it off of the area and angle of the droplet (Lee 1987). Both of these methods are acceptable for liquid samples but do not work when looking at solids.

Moving away from liquids, a method to determine surface hydrophobicity can be taken from packaging that allows one to find the surface hydrophobicity of a food product that is homogenous. The Dynamic contact angle measurements can be done by dropping water droplets onto the surface of the food product and measure the angle and spread of the droplet on the surface of the food product. While both of these methods are acceptable, one can also use a scanning electron microscope to exam the surface to look at pore dimensions, thus be able to estimate how hydrophobic the exterior layer is (Rodionova et al 2010).

Analytical Methods: Colorimeters

Color is read using an absorbance of solids or liquids in a mechanical system, and colorimeters are used to mimic human perception of color or color change.

Colorimeters come in many size shapes and forms, while the guiding principle is that a short burst of ultra-bright white light is emitted, reflecting back to the system, and the color is run through red, green, and blue filters that are meant to match the colors the human eye perceives (Choudhury 2014). The standard output for these new systems is in terms of L^* , a^* , and b^* . L^* indicates the difference between light and dark. As the number becomes higher, the lighter the substance, the lower the number indicates that the substance is darker (Hunter labs). The A^* indicates how red or green the product is. Similar to L^* , the higher the a^* it means the substance is redder in nature, while the greener pigments are negative in numbers. Lastly, b^* indicates how yellow or blue the substance is; the higher the number, the more yellow it is, and the more negative the number represents blueness as shown in Figure 3 Colorimeter Color L^* A^* B^* Visual Explanation. Comparing two or more samples, δL^* , δa^* , and δb^* are used to indicate the difference between them, and an δE^* is used to indicate the total difference between the samples. To determine δE^* , the following equation is used.

$$\delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

Equation 1: colorimeter Delta E formula for change in color

Based on this color formula, one can determine if the product or system has changed in color, even when it may not be noticeable by the human eye. The further the δE^* value

is from 0, the more noticeable the differences should become over those with small numbers (Becker 2016).

Analytical Methods: Water Activity

Water activity, although it is not one of the top two issues with bar hardening, is a contributing factor since the water will migrate from higher water activity areas to lower when looking at protein bars that are not homogenous with no layered migration barrier in place (Li et al 2008). While higher protein bars have an approximate ration of 30:30:40 protein, fat, and carbohydrate syrups, respectively, creating a product that can be extruded and inclusions added if desired. Scientists are looking for alternatives to reduce bar hardening in protein bars. One such approach is the use of hydrogenated oils, like cottonseed, mixed with whey proteins to create a hydrogenous whey protein isolate. When Hydrogenated oils are added with high fructose corn syrup like vegetable shortening, the resulting product did create a softer bar but still had mallard browning reactions and did not remove all hardening from the product. The product's shelf life went from a three to month shelf life to something closer to nine months before becoming inedible (McMahon et al 2009). The current methods to decrease bar hardening are focused more on the sugar alternative and the use of alternative proteins than WPI's and not looking at disrupting the protein's ability to migrate by changing the forms of fat.

Tables and Figures

Table 1 History Leading up to Current Product Market

Historic timeline leading up to nutrient-dense protein bars	
3,200 BC	Pemmican
1863	Granola®
1960's	NASA® product
1964	Revitalized granola trend
1972	Quaker® acquired granola
1974	Modern Granola bar
1986	PowerBar® introduction
1990's	Slimfast® bars
2018	Solder Boost

Table 2 Protein Bars on the Market with Examples

Current protein bar market with activity levels				
Category	Snack bars	Nutrition bars	Performance	Weight loss
Balanced nutrients	Granola	Fruit filled product	Extruded high sugar bars	Low calorie, low fat, high protein
Product example	Chewy	Cereal bar	PowerBar®	Slimfast® bar

Table 3 Common Bar Market Examples with Macronutrients

Common bar market examples					
Product name	Chewy® chocolate chip	Cereal® bar	Clif® bar Chocolate brownie	PowerBar® chocolate	Solder boost
Protein content	1g	1.7 g	9 g	9 g	30 g
Fat content	3.5 g	3.2 g	5 g	3 g	40 g
carbohydrate content	17 g	26 g	44 g	45 g	31 g
Total kcal	100	120	250	220	490
Type of bar	Snack bar	Nutrition bar	Nutrition bar	Performance bar	Protein dense performance bar

Table 4 History of Military Meals

History of Military Meals		
Dates used	Name of meal	Contents of meal
1785 -1813	garrison ration	Freshly cooked meat, vegetables, and bread
1785-1865	Spirit ration	Freshly cooked meat, vegetables, and bread, 4oz whiskey or rum
1813 - 1917	Canned food	
1907 - 1914	Emergency ration	3 oz cakes flavored, 3 1 oz bars chocolate, salt, and pepper
1914 - 1918	Trench ration	Canned meat (salmon, corned beef, or sardines)
1917 - 1937	Reserve ration	Canned meat (corned beef or pork and beans), 16 oz bread or biscuits, coffee.
1938 - Current	A ration	Fresh or frozen food prepared in dining halls (Varied products)
1938 - Current	B ration	Canned foods cooked in field kitchen (varied products)
1938 - 1958	C ration	Canned meal
1938 - 1948	K ration	Three meal pack all canned, Veal, ham and eggs, biscuit. Lunch meat, canned cheese, biscuits, eight cigarettes. Dinner, Canned meat (3 different types), candy bar.
1938 - current	D Ration	Concentrated chocolate, with other ingredients to provide energy
1958-	M	The M (meat) unit and B (bread) unit M unit could be canned meats or meat and pasta dishes. B unit was crackers, beverage powder, oatmeal found with a Breakfast pack
1958-1980	Wet MCI	Canned meals with 12 different meal options
1975 - Current	MRE/ MRE XVIII	24 different meal options including vegetarian options

Table 5 Common Bar Market Examples with Macronutrients

Common bar market examples					
Product name	Chewy [®] chocolate chip	Cereal [®] bar	Clif [®] bar Chocolate brownie	PowerBar [®] chocolate	Solder boost
Protein content	1g	1.7 g	9 g	9 g	30 g
Fat content	3.5 g	3.2 g	5 g	3 g	40 g
carbohydrate content	17 g	26 g	44 g	45 g	31 g
Total kcal	100	120	250	220	490
Type of bar	Snack bar	Nutrition bar	Nutrition bar	Performance bar	Protein dense performance bar

Table 6 Lifestyle, Based on Activity Level and the Associated Bar Suggestion

Lifestyle with activity level and bar suggestions				
Lifestyle	Sedentary lifestyle	Moderate activity	Intense activity	Extreme activity
Kcal needed	1900-2100	2200-3000	3000-4000	5500+
Activity level	Sedentary office	Moderate activity	High activity	Intense weeks- long activity
Example activity level	Walking around office	Light Workout daily	Heavy workout daily	Workouts exceeding 7 hours a day
Bar type needed	Meal replacement bar	Performance bar	Performance bar + snack	Nutrient-dense protein bar

Table 7 Structure and Stability Ranking

Structures and Information about Cocoa butter		
Structure	Melt point in °C	Stability ranking
Gama	16-18	1 (least stable)
Gama prime	22-24	2
Alpha	24-26	3
Alpha prime	26-28	4
Beta	32-34	5
Beta prime	34-36	6 (Most stable)

Table 8 Cocoa Butter Alternatives

CBE CBS and CBR's and important information		
Cocoa butter substitutes	Melt point	Lauric, Non-lauric, or Allied*
Coconut oil	28.9°C	Lauric fat
Palm kernel oil	20.0-54.0°C	Lauric fat
Shea butter	32.0°C	Allied
Mango kernel fat	32.0-43.0°C	Allied
Cottonseed oil	-1.0°C	Non-lauric

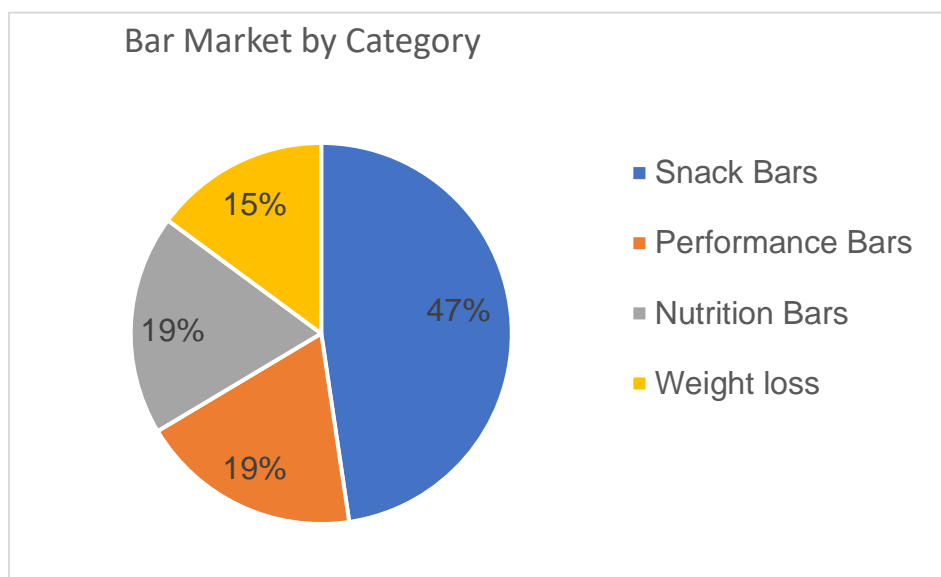


Figure 1 Bar Market Categories Broken Down by Percentage

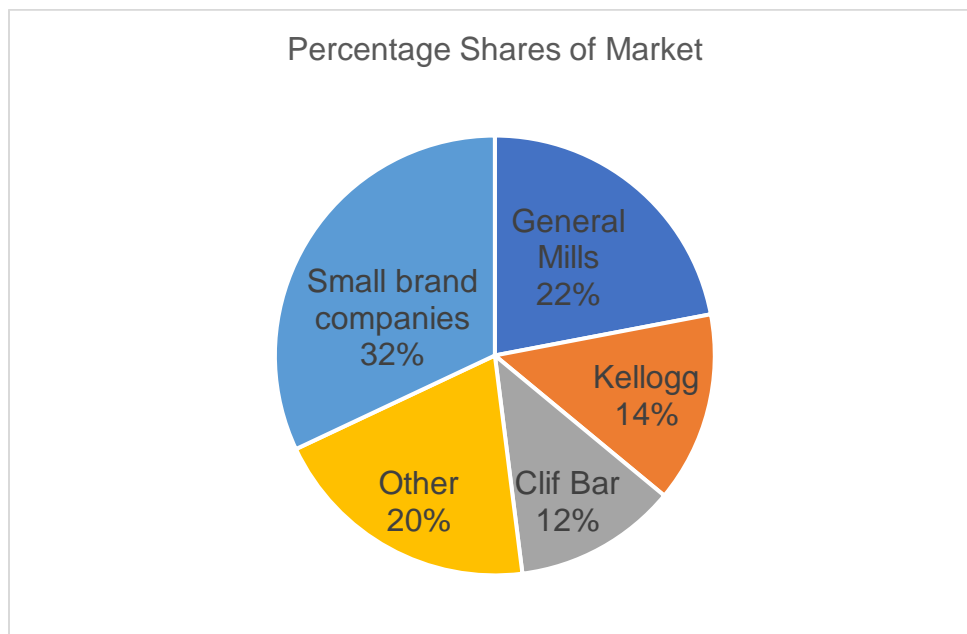


Figure 2 Shares of Sales by company over 52 weeks in 2019

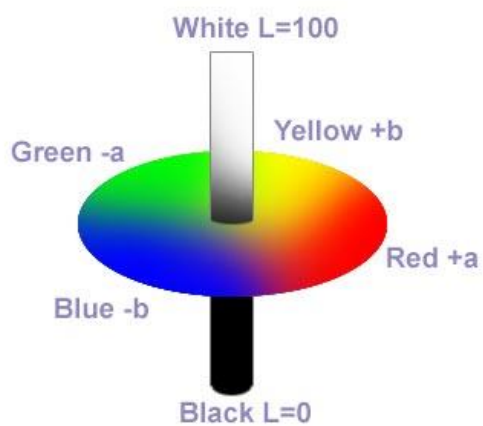


Figure 3 Colorimeter Color $L^* A^* B^*$ Visual Explanation

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Chapter 2: Development of a Shelf-Stable Nutrient-Dense Protein Bar

Abstract

Shelf life is a limiting factor to otherwise popular protein bars due to protein aggregation and migration or loss of other nutrients. Rigorous military training in extremely cold conditions may require in excess of 6,000 kcal per day, but current rations are wasted because they are bulky, heavy, or not appealing. This project aims to address bar hardening and flavor issues to create energy-dense, shelf-stable bars in various flavors while standardizing nutrient profiles. A review of the protein bar market indicated chocolate as the most common flavor. Savory flavors are less common and have shorter life spans in the market. Unlike the existing protein bars that are high in sugar and low in fat, this project focuses on a high-fat, high-protein, low water activity bar to maximize energy density and extend shelf life. A variety of flavors, including chocolate and cheese, were created to limit consumer boredom. While other flavors are being developed, sensory acceptance of the chocolate protein bar has shown positive results with military and veterans. The most stable fat/protein ratios with high protein content were determined from different combinations of dairy protein and fats. Palm kernel oil (PKO), cocoa butter, coconut oil, and canola oil were evaluated in combination with milk protein isolate, a dairy protein blend, whey protein concentrate, and two whey protein isolates (WPI). The development of this unique nutrient-dense protein bar went through over 150 different formulations over four years. The ideal fat protein mixtures for military personnel and extreme athletes are combinations of WPI's with PKO or cocoa butter, yielding an ideal protein bar that provides high nutrient density, and extended shelf life with no hardening.

Background Information

Product Development

Product development has been an essential part of innovation and the creation of new and exciting products. Creating new products can be seen as a challenging task, and many different processes have been created to help pave the way to innovation. One such process is the Cooper Stage-gate process, invented in the 1990s focusing on being a flexible roadmap to shorten the timeline on new product development (Aramouni and Deschenes 2018). This method uses five steps, 1) concept; 2) feasibility; 3) business case; 4) launch coupled with the post-launch review. In comparison, this method had a large focus on fitting into the market and how feasible the product was. The New Product development process takes what Cooper did and creates a new method focusing on eight steps 1) idea generation; 2) idea screening 3) concept development and testing; 4) Marketing strategy development 5) business analysis; 6) product development 7) test marketing 8) commercialization (Fuller 2011). This method has a better organization and flows to it without making the process too stiff and rigid. While both methods have valid merit to create a good product, the same basic premise; to a concept, see if it is feasible, review current marketing, create a strategy or business model and launch the new product (Thomas 1993; Head 2013). Understanding the market a new product is going to be entering is a key step in the product development process.

Protein Bars

Protein bars are one sub-category from the bar market, but while these are a portion of the market, they get placed in with performance bars. The branches of the bar

market are snack bars, nutrition bars, performance bars, weight loss bars, as displayed in Figure 4 Bar Market by Category no Separate Section for Protein Bars. The bar market has begun to crosslink between performance bars and nutrition bars creating as products like a Clif® bar and power bars both contain 9 g of protein, Clif® bar has a fat content of 5 g, and the PowerBar® contains 3 g of fat, and carbohydrate content of Clif® bar with 44 g and PowerBar® has 45 g of carbohydrates. These two products and others are presented in table 7, showing the amount of protein and fat in each of the products by percentage.

Processing Methods

The process of extrusion places the dough, or future product, inside a machine that takes two screws and applies temperature and pressure to push out the product and create a paste. Based on the temperature, pressure, and mold at the end, the product can have a light, airy product like cheerios or nougat candy filling or something heavy and dense like an original power bar (Minifie 1999).

The second processing method is glued, this method takes a product again in a hopper and is placed on a conveyor belt with walls on the sides, and it travels down where it goes through. A bunch of rollers slowly presses it to the desired thickness and pushing the product to touch the sides of the railing (Minifie 1999).

The third method has to do with chocolate products. This method requires the product to go from a liquid state into a mold. The mold can be made out of many different materials, and some examples are silicone, cornstarch, ceramic, and wax paper. Once the product is placed into the mold, it will start to cool or set, depending on the process, and if tempering was required, the product might need to be placed in a

blast freezing system to cool quickly or gradually temperature lowering chambers (Beckett and Royal Society of Chemistry (Great Britain) 2008).

The fourth process is used with either glued or extruded products, and it's called enrobing. The product is placed on a conveyor belt with lots of holes in it and driven under a fountain of chocolate or chocolate-like product, creating a coating on the outside while the excess coating material gets collected and reused.

Military Application

The military has had an issue sustaining soldiers when they are on the move in extreme weather conditions. While meals ready to eat (MRE) are used in forwarding operating bases and while on motorized patrols outside the wire, also known as safe zones. MRE's have their own issues. The first issue with MREs is Field stripping; this is the act of removing anything unwanted from the meal (Hawver 2006; Stilwell 2015; Lunn 2019). This could be the extra packaging like the dark outer packaging telling us what the meal is, cardboard, and undesirable meal items. Sometimes people will trade food items they do not want for the ones they do. One example of this would be trading the cheese sauce pouch for the peanut butter pouch. Sometimes the only item people keep is the candy or dessert item, and everything else goes in the trash or gets burnt in a fire (Dominguez et al 2018).

The field stripping issue creates a new issue of calorie deficit. If someone eats 3 MRE's every day, they are going to consume between 3,500 and 4,000 kcal each day, with an average MRE being 1,250 kcal per meal (Gonzales 2005; Hawver 2006). Now, if the soldier doesn't eat all the food inside the MRE, that is an issue by itself, but in extreme hot or cold weather, Caloric needs become higher. In some extreme cases, it

exceeds 6,000 kcal per day to just maintain one's body weight (Beals et al 2019). In these extreme conditions, when someone is marching or working with moderate to heavy exercise is how one gets to such extremes as 6,000 kcal.

The military is looking to solve issues with calory deficit not by changing MREs but by adding new options instead of providing a fourth meal each day. The goal of the project is to create a shelf-stable nutrient-dense protein-fortified bar. They currently use the paratrooper bars and the Hooah® bars, also known as the soldier fuel bar. The Hooah® bar contains 10 g of protein, 9 g of fat and 40 g of carbohydrates, and about 280 kcal. The alternative choice is the first strike nutritious energy bar with 10 g fat, 4 g of protein, and 46 g of carbohydrates from the total 65 g and 280 kcal. Noting these bars are not extremely temperature stable, but they do provide shelf stability for two-plus years with some hardening.

Materials and Methods

Market Analysis

Purchase multiple types of protein bars from many sources with a wide range of flavors, from sweet to savory. These should be purchased from grocery stores, convenience stores, health food stores, and online retailers. Products should be accepted with either supplement labels or ingredients labeling as shown in **Table 9**. Taste each product, recording all ingredients, with photos of each product. The taste should cover texture, flavor, and appearance. If possible, determine processing methods. For this system, use a stand mixer with paddles to create shear forces on the dough, with slab rolling for ideal processing.

Product Creation

Create an ideal product from market analysis and find ingredients that will meet the requirement. Assemble test products from the common ingredients or, based on desired characteristics, use processing methods that seem appropriate. Taste each bar with a team to determine if the product is getting close to desired taste, texture, and appearance. Create test bars using lipids from **Table 10** Cocoa butter Equivalents, Substitutions, and Replacements Melt Points and proteins from **Error! Reference source not found.** to find the best mixture, combinations of fats might also be required to get desired flavor and texture.

Market Testing

Determine test market based on age, sex, gender, and any other requirements. Create a sensory ballot using 9 and 5 point hedonic scales with desired characteristics, with one or more test products. Run sensory testing with a ballot and selected group to determine product acceptability.

Results

Market Analysis

Table 12 shows a list of the protein bars currently on the market. The ProtiDIET[®] High Protein Chocolate Dream Bar comes in with 30% protein, 40% fat, and 30% carbohydrates. It requires at least two types of emulsifiers to provide it with the same product, but it does not hold up in extreme temperatures because of its use of just cocoa butter, chocolate liquor, and soy. The product even looks like a chocolate bar. Another downside is that this product uses a lot of non-chocolate bar products. This is maltitol, polydextrose, tapioca starch, Butylated hydroxyanisole, butylated

hydroxytoluene, and polyglycerol polyricinoleate. Adding these ingredients should not be seen as a bad thing as BHA and BHT are just synthetic antioxidants to help prevent oxidation in food products, while maltitol is a sugar alcohol used to provide sweetness and does not cause browning, and polydextrose is another synthetic polymer of glucose. Both of the sugar alternatives do provide a downside of off flavorings, and people tend to say they have a metallic flavor that lingers on the tongue. Looking past the off-flavors, they did create a good product for the everyday consumer, but they use at least two sugar substitutes that do not provide the same energy as glucose would readily available and do not hold up to extreme temperatures. The only conclusion is that the ProtiDIET® High Protein Chocolate Dream Bar does not meet up with the new product developed displayed in .

Protein bars create line extensions to reduce fatigue from overconsuming a single flavor. Some typical flavors are peanut butter, peanut butter and chocolate, jalapeno chocolate, and generally, they are sweet. Savory flavored products rarely seem to last in the bar industry. While people want savory flavored protein bars, the end result leaves people getting flavor fatigue faster than sweet flavors.

Product Developed

The new product soldier on being 65 g, with 320 kcal per serving. Of that, 19 g is fat, 18 g is carbohydrates, and 19 g is protein. At the same time, the product is 30% protein and 30 % fat with a mixture of CB and PKO to achieve desired melt point above 55°C. The protein selected was provon as it had the highest protein content, oat flour used for digestion, Cocoa liquor used for flavor, as was salt.

Market Testing

Market testing was done by a Preliminary sensory study, Figure 2, indicated that in a small sample, the chocolate flavor was too little, with 55% of the consumers saying this, while the texture of the product was just about right by 55% and the desired sweetness was just about right according to 62% of the taste testers. At the same time, the overall appearance had a mean of 6 with a standard deviation of 1, and overall likeness had a mean of 6 with a standard deviation of 2.

Discussion

Market Analysis

Many of the ingredients had been determined by what chocolate bars use, what is the most popular protein type for protein bars, and what is an excellent way to make it calory dense. Traditionally chocolate bars use cane sugar during a grinding step to break the size down and incorporate it into the melting fat and cocoa solids to create a semi-sweet to a sweet product. When adding cocoa liquor, protein, oat flour and then add dry sugar, the product does not have enough liquids in the mixture to even make a reliable product shown in Figure 6 Dark Chocolate Bar. By changing out cane sugar for inverted sugar, provides the same sweet flavor, adds moisture while still keeping the water activity below 0.68, making the product shelf-stable and is not a significant concern for most microbial growth. By adding inverted sugar, it increases browning reactions when placed in extreme temperature conditions. By replacing invert sugar with 60%/40% sucrose/water, the products kept the water activity down as the water is bound to the sucrose, adds the required moisture, and desired sweetness while limiting

the browning reaction. Savory flavors have been developed but, browning reactions occur when placed at temperatures of 55°C.

Extrusion was not successful because it adds too much pressure, thus causing the fat to separate from the solids of the bar. The nutrient-dense protein bar requires the slow-rolling method as used with glued products, even though it is not a glued product and is too thick for pouring into a chocolate mold.

Market Testing

Preliminary sensory analysis showed that the texture was just about right, as was the sweetness, while the chocolate was too low in flavor. The chocolate flavor being low as a bad thing but is not factoring in flavor over time or even the number of bars that get consumed over the course of a week. A single chocolate bar is good, but it can be hard to eat three to five full-size chocolate bars in the span of three days without being tired of the flavor. This analysis also did not factor into that some of the people who did the evaluation had been competitors and intentionally provided low or negative responses to end the product before making it to market.

Conclusion

The market analysis indicated that most products did not have both high protein as well as high-fat content and that a new type of product market is present that creates nutrient-dense products that are shelf-stable. Chocolate or sweet flavors are more favorable than savory in the protein bar market. The new product that was developed has both high protein high fat that meets a new area in the market. The new product has a combination of Cocoa butter and PKO to create a higher melt point preventing the product from melting in extreme weather conditions like Afghanistan.

Future Work

Future work should include breakpoint testing on accelerated shelf-life products. Extensive sensory analysis with soldiers from mass-produced products to help refine the final product. Lastly, production cost analysis of ingredients and processing equipment required for full scale-up in production.

Tables and Figures

Table 9 Protein Bar Market Research with Clams, Protein, and Fat Content

Brand	Components	Boasts	Protein content	Fat content
MySmart® Bars	Sorbate suggests mold problems		20%	18.4%
Vegan Protein Bar®	Sacha Inchi- star-shaped nut, high in omega's (H3)	Vegan, Paleo, "lava" middle, soy-free, non-gritty	40%	15.0%
Quest bar® (chocolate brownie)			33%	11.6%
Gatorade Whey Protein Recovery Bars	BHT --> Butylated hydroxytoluene, prevents oxidation		25%	16.3%
ThinkThin® Dark Chocolate Protein Bar			33%	13.3%
Paratrooper bar(chocolate)			6%	13.8%
ProtiDIET® High Protein Chocolate Dream Bar	polyglycerol polyricinoleate- an emulsifier, even though soy lecithin is already used/included	It looks like a chocolate bar	30%	40.0%
MuscleTech® Mission1	Erythritol- sugar alcohol		33%	10%
The Bar Counter®: Chocolate Brownie Crunch		raw, clean protein	20%	10.5%

Table 10 Cocoa butter Equivalents, Substitutions, and Replacements Melt Points

CBE CBS and CBR's and important information	
Cocoa butter substitutes	Melt point
Coconut oil	28.9 °C
Palm kernel oil	20 - 54.0 °C
Shea butter	32.0 C
Mango kernel fat	32.0 – 43.0 °C
Cottonseed oil	-1.0 °C
Cocoa Butter	37 °C

Table 11 Whey Protein Information

Whey Protein name	% Moisture	% Protein	Protein type
Barpro 288	4.14	90.36	Dairy protein blend
Barpro 585	5.29	86.14	Milk protein isolate
Avonlac 180	4.00	80.79	Concentrated whey protein
Provon 190	3.61	94.02	Whey protein isolate
BevWise A100W	2.36	84.10	Whey protein isolate

Table 12 Market Analysis with New Product Present

Brand	Components	Boasts	Protein content	Fat content
MySmart® Bars	Sorbate suggests mold problems		20%	18.4%
®	Sacha Inchi- star shaped nut, high in omega's (H3)	Vegan, Paleo, "lava" middle, soy free, non-gritty	40%	15.0%
Quest® bar (chocolate brownie)			33%	11.6%
Gatorade® Whey Protein Recovery Bars	BHT --> Butylated hydroxytoluene, prevents oxidation		25%	16.3%t
ThinkThin® Dark Chocolate Protein Bar			33%	13.3%
Paratrooper® bar(chocolate)			6%	13.8%
ProtiDIET® High Protein Chocolate Dream Bar	polyglycerol polyricinoleate-emulsifier, even though soy lecithin is already used/included	Looks like a chocolate bar	30%	40.0%t
MuscleTech® Mission1	Erythritol- sugar alcohol		33%	10.0%
The Bar Counter®: Chocolate Brownie Crunch		raw, clean protein	20%	10.5%t
Soldier On®: Chocolate Brownie		Nutrient dense, shelf stable	27.85%	29.2%

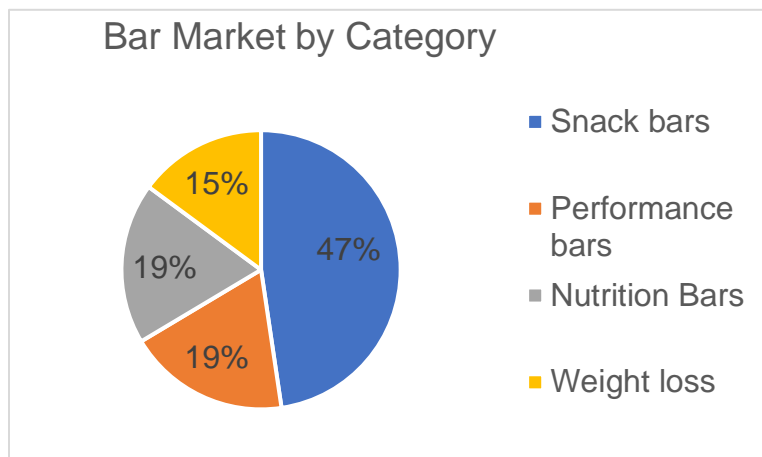


Figure 4 Bar Market by Category no Separate Section for Protein Bars

Acceptance testing Chocolate bar preliminary

Variable	Minimum	Maximum	Mean	Std. deviation
Overall appearance	3	8	6	1
Overall liking	2	8	6	2
Overall flavor	2	8	6	2
Overall Chocolate	3	8	5	2
texture	3	7	4	1
Sweetness	4	8	6	1

Variable	Level	Frequencies	%
Jar chocolate	<i>Too little</i>	15	55.56%
	JAR	12	44.44%
	Too much	0	0.00%
Jar texture	Too little	3	11.11%
	<i>JAR</i>	15	55.56%
	Too much	9	33.33%
JAR sweetness	Too little	10	37.04%
	<i>JAR</i>	17	62.96%
	Too much	0	0.00%

Figure 5 Preliminary Acceptance Testing Dark Chocolate Bar



Figure 6 Dark Chocolate Bar

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**Chapter 3: A Method for Determination of Unbound Fats in Protein Bar
Formulations Compared with Isolated Fat/Protein Combinations to Optimize Shelf
Life**

Abstract

Protein bars form a growing part of the snack food industry, but the shelf life is typically limited by bar hardening due to protein aggregation related to the migration of other nutrients. Addressing these issues will extend protein bar shelf life while continuing to maintain nutrient density for military personnel and extreme athletes. We developed a method to examine the interaction of fats and proteins in bars and in simple mixtures in order to address lipid/protein interaction and lipid migration. Mixtures of dairy protein and fats were created in order to determine ideal fat/protein ratios. Fats tested were palm kernel oil (PKO), cocoa butter, coconut oil, and canola oil. Proteins included milk protein isolate, a dairy protein blend, whey protein concentrate, and two whey protein isolates (WPI). Fats were liquified, mixed with protein at 0/100 up to a 50/50 ratio by weight, and allowed to solidify. Solidified pellets were suspended in filters placed in conical tubes at a temperature of four degrees Celsius above the melting point of the fat for twelve to twenty-four hours. The weight of melted fat that passed through the filter was then determined. At 50/50 ratios, the combinations of cocoa butter and PKO with WPI and whey protein concentrates demonstrated 1% and 3% fat loss, respectively. While a ratio of 59/41% lipid/protein respectively, cocoa butter and PKO with WPI continue to have the lowest residual fat at 14%, while the highest is coconut oil and milk protein isolate at a residual fat content of 29%. Low residual fat is between one and three percent residual in a 50%/50% matrix, while low-fat retention is anything

above 14% residual fat. The dairy blend and milk protein isolate have low-fat retention with saturated fats but have increased retention with canola oil. Coconut oil has a high residual fat of 25% with the milk protein isolate that contains both casein and whey proteins. The ideal fat protein mixtures for military personnel and extreme athletes are WPI's and with PKO or cocoa butter. By using these two fats with the whey protein isolates, it creates an ideal matrix for protein bar stability, preventing bar hardening and long shelf life while keeping nutrient density.

Introduction

Protein bars consist of some form of sugar or sucrose, protein, lipid or fats, and water. While this seems straightforward but the concentration of these major ingredients has a direct reflection in shelf-life (Li et al 2008; McMahon et al 2009; Keefer et al 2020). Protein bars come in a range of protein content from as low as 6% to as high as 40%, with an average of 20% protein content. In most protein bars, the amount of fat in the product does not exceed 20% of the total product, with a few exceptions. Protein bars with a low percentage of fat usually have a very high percentage of sucrose or sweeteners. While this approach works well for most products to achieve the nutrient content desired but by increasing the amount of lipids is the best method I create nutrient-dense products. While creating a product with nutrient density is a challenge of its own, optimizing the shelf life that is stable above temperatures of 54°C is a whole new adventure.

Ingredients

Lipids

With a product that needs high-fat rations, a number of fats should be considered. Cocoa butter (CB), palm kernel oil (PKO), Coconut oil, and Canola oil was considered for different properties they possess (Jahurul et al 2013). CB was first looked at because the idea originated from eating chocolate and thinking this requires minimal packaging considerations and has a long shelf life at 22°C. At the same time, PKO is looked at because it accepts temperatures above 55°C and Coconut oil because it is a current trend and has been known to be a substitute for CB in chocolate products (Biswas and others 2016). Lastly, Canola oil was used because it is not saturated and has a melting point below 0°C. BC most stable crystal structure is Beta prime (β') but undergoes a transition from Beta (β) to β' within three weeks from original formation being set (Vaack 1960). While PKO has a preferred crystal structure, that is β , and Coconut oil has a primary crystal structure of β' . When mixing two or more of these fats together, they greatly change the structure and melting points of the product but also greatly increases the chance of bloom (Limbarido et al 2017).

Whey Protein Isolate

Whey Protein Isolate (WPI) was determined to have the ideal properties for this particular protein bar based on its interactions with CB, PKO, Coconut oil, and Canola oil. This particular WPI has a protein content of 94% protein, with 3.6% moisture. Other Whey protein blends considered had been Dairy protein blends, milk protein Isolate, concentrated whey protein, and a second WPI (Adamson 2011). Each of these had a protein content above 80 percent and moisture below 6 percent (Hogan et al 2012).

Shelf life can be determined by multiple methods; the first method the slowest method, and that is by physically setting the product on a shelf and leaving it alone; this could take years. Another such method is a more accelerated method that uses a heated chamber at two different temperatures over a much shorter time. Lastly and the least accurate method is by literature. Using literature of similar products on the market, one can get an estimation of how long it will be acceptable. This last method is generally only done internally when they have a database of similar products to get a rough estimate of the product's shelf-life. The color change or browning represented on a protein bar can help indicate the bars' acceptability as the appearance has been connected to the likeability of food products. One such method to determine color is using a color meter (Steele 2004).

Color meters are similar to spectrophotometers, except they base the change on perceived color changes (Choudhury 2014). This type of color change is based on the human eye and the amount of light that passes through the object and is not the same as spectrophotometry measures wavelengths (Becker 2016).

To determine the optimal fat and protein combination for a shelf-stable product at temperatures up to 55°C optimizations of unbound fat to prevent fat leach, and an accelerated shelf-life study was quantifying vitamin C degradation and color change over 30 days.

Methods

Simple Unbound Fat System

To determine the shelf life of the product based on the amount of bound or unbound fats left in the system, a simple method was created. The method takes 0 g to 10 g of lipids and melts them in a 50ml conical tube, and adds protein powder at a

concentration of 0 to 10 g, in one-gram increments, to determine optimized protein cohesion. Anything that cannot become liquid to paste consistency will become the upper limit. Examples of this are lipid becomes encased in protein powder and has a dry, crumbly texture and appearance. Cocoa butter with more than 60% WPI did not create a pellet or slurry. Freeze the pellet for at least 30 min and for unsaturated fats for 1 hour in a -40°C freezer or colder. Remove pellet from 50 mL conical tube and wrap in miracloth® and suspend in a conical tube, ensuring the lid is on securely-Place samples in an incubator at temperatures exceeding the melting point of the fat by at least 1°C. Remove samples 12 h later and immediately centrifuge at 200 g for 3 min: freeze samples and measure weights of now two pellets.

Complex Unbound Fat System

The complex residual fat method is a variant of the above method that can be done with the protein bar formula or other multi-ingredient products and replacing the original fat with alternative fat choices.

Shelf-Life

A shelf-life study was conducted with three incubators set to 22°C, 39°C, and 55°C with control of timepoint 0 samples stored in the freezer at negative 40°C. The samples frozen are placed in the freezer the same day as samples placed in incubators. Incubated samples had been created over three days, with three samples made of each variant on each day, and placed randomly in incubators for either 15 or 30 days. 27 samples have been prepared over three days with three samples of each treatment processed each day and randomly assigned either 15 or 30 days and randomly assigned 22°C, 39°C or 55°C. All samples are stored in the negative 40°C freezer after

the appointed end time until analysis can be done. Vitamin C was added to the protein bars for titration analysis to determine degradation over time.

Colorimeter

Colorimeter analyses are run on a hunter lab, Colorflex[®] EZ coffee color meter. Each sample was removed from its package, placed on the color meter with three readings from each bar to create an average color. This is done after all samples had been removed and frozen for uniformity and compared to time point 0. A Delta(δ) E value was then used to determine the amount charged over the given length of time using the equation

Vitamin C Titration

Titration was run on a Thermo scientific orionstar[®] T940 Series Potentiometric titrator, using a modified AOAC 967.21 method. Five g of protein bar with three rounds of 5 mL Metaphosphoric acid- acetic acid solution by Vortexing for 1 min and centrifugation at 7,900 g for 12 minutes, creating a 15 mL supernatant. Extract supernatant and kept in a conical tube under nitrogen and in a dark place, or Freeze if stored for an extended amount of time. Titrate with Indophenol standard solution, and use ascorbic acid solution using 1 mg/mL reference (Nielsen 2017).

Results

Simple Residual Fat Matrix

In figure 3 shows the residual fat separation amongst all of the Canola oil samples had been below 1 g residual fat with all of the different protein types. While the coconut samples had the largest residual fat separation when combined with the bar pro[®] 585 protein. Barpro[®] 585 also had a higher separation rate with PKO and CB than

with the other protein types, except when combined with Canola oil. Samples that are below 0.5 g separation can be seen as ideal combinations under this simple mixture.

Complex Residual Fat Matrix

The complex mixture had no significant difference between any of the systems. All samples had less than detectable residual fat loss when stored at 55°C. These same results had been observed even when oscillating between 22°C and 55°C every other day for 15 days. Complex treatments had been produced with CB, PKO, Canola oil, and a modified version of CB. Modified CB was created by taking 50% cocoa solids and 50% cocoa butter, while the original used cocoa liquor.

Shelf life

Titration

The standard had an average titration of 12.13 mL, with the average of the blank at 0.36 mL. The following calculation was used to determine the amount of vitamin C retained from a 100 g protein bar. X is the average mL from standard (12.13), B is the blank (0.36), F is mg ascorbic acid equivalent to 1mL of indophenol solution (0.16488), E is the size of the sample used in extraction (5 g), V is the total volume of supernatant (15mL), and Y is the total mL titrated (7mL). The following will become fixed B, F, E, V, and Y after the initial samples. The X value after standards will become the amount titrated for any given sample.

$$\text{Vitamin C} = (X - B) * \left(\frac{F}{E}\right) * \left(\frac{V}{Y}\right)$$

Samples stored at room temperature or 22°C showed the least degradation over both 15 and 30 days between all the samples. PKO and Avalac® had two samples represented in 15 days at 22°C with 37.2 mg retained and with 22.3 mg retained over 30

days. Cocoa butter and provon[®] had zero samples at 15 days and two samples at 30 days with 21 mg and 6 mg vitamin C retained. Canola oil and Barpro[®] had two samples at 15 days with 20.6 and 28.2 mg retained and one sample at 30 days retaining 17.8 mg.

The PKO Avalac[®] had three samples stored at 39°C, one of those at 15 days and the other two at 30 days. At 15 days, PKO Avalac[®] retained 5.0 mg while at 30 days retained 1.9 mg, and 11.0 mg of vitamin C. Cocoa butter and Provon[®] had three samples at 39°C, one sample for 15 days, and two at 30 days. Cocoa butter and provon[®] 15 days had retention of 11.0 mg and 30 days retention of 1.9 mg and 2.4 mg of vitamin C. Canola oil and barpro[®] had three samples stored at 39°C, two at 15 days and one at 30 days. The retention of samples at 15 days is 35 mg and 3.4 mg, while the 30 day sample had 11.0 mg retained.

PKO and Avalac[®] had three samples at 55°C, with one sample at 15 days and two samples at 30 days. PKO and Avalac[®] retained 1.0mg of vitamin C at 15 days and .8mg for both samples for 30 days. Cocoa butter and Provon[®] had three samples at 55°C, with all three samples at 15 days. They had an average of 1.4 mg with a range between 0.4mg and 2.5 mg. Canola oil and barpro[®] had three samples at 55°C, two of those at 30 days and one at 15 days. The sample at 15 days retained 1.2 mg of vitamin C, while the two samples at 30 days retained 0.8mg and 0.4mg of vitamin C.

Colorimeter

The colorimeter δE calculation and E value are represented by the following equations:

$$\delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

$$E_a^* = \sqrt{(L_1^*)^2 + (a_1^*)^2 + (b_2^*)^2}$$

The Delta E, when compared to Storage temp and storage time of either 15 or 30 days, the F probability at 15 days is 0.2483 while at 30 days is 0.8870. This indicated that the time spent at the given temperature does affect the total color change of the products in question. When looking at the storage length of PKO and Avonlac® 180, the color change and an F probability of less than 0.7246 for a two-sided T-test. With the Original formula being Cocoa butter Provon® 190, it has an F probability of 0.0309. Lastly, the Canola oil with Barpro® 585 had an F probability of 0.3200. There is a noticeable difference in the PKO Avonlac® combination and the least noticeable change in the Cocoa butter and Provon® 190. Looking at just the δE by sample type at each temperature, the difference between all samples at 22 °C only has a probability of 0.0172, and at 39 °C 0.060 with the least difference at 55 °C with a probability of 0.0001. This shows that the storage temperature did not affect the color change of the protein bars.

For all protein bars, the biggest change was with the L value, as that indicates the change in light and darkness. While a positive number means it got lighter, and a darker number is represented with a negative. Most samples got darker than the original point, but values did not indicate that the storage length was the contributing factor. The sample with the largest variability in color is PKO and Avonlac® 180 with a standard deviation of 2.026 for all nine samples pre-storage with a coefficient of variation of 4.575%. Cocoa butter provon® 190 mixes had a standard deviation of 0.9130 with a

coefficient of variation of 2.081%, and the Canola oil with Barpro® 585 had a standard deviation of 0.9037 and a coefficient of variation of 2.104%.

Discussion

Residual Lipid Values

Residual lipid values have not been researched, while hydrophobicity has been looked at by the surface tension of a water droplet measured by dynamic contact angle between the droplet and the object for solid surface only products or hydrophobic gel chromatography for liquids. A new method was needed to find out if any residual fat was present or to determine the ideal amount of fat in a product. Sugars and protein interactions had been presented by Tran, 2009 and other authors. Fat being the primary connection point with whey has not been present.

Simple Residual Lipid Matrix

The Simple residual fat matrix showed that PKO and Avalac® 180 was the best combination and had the least residual fat, while cocoa butter and provon® 190 was close to the original formula, and while canola oil did well, it had a very high variability as a result of it being an unsaturated fat, but when combined with barpro® 585 being the worst at binding with saturated fats. The complex matrix did not prove to add any new information but showed other added ingredients help for a true good binding product. No noticeable changes to the bars after extended time in the incubators or with oscillating had occurred.

Colorimeter

The Colorimeter data indicates that the biggest connection from shelf life is the amount of time is the factor, shows that time when connected to higher temperatures

has a bigger chance of showing color change over the course of 30 days. This also indicates that this model could help note that over 30 days, it represents. The standard deviation and coefficient of variation of the samples pre-storage indicated that all the bars had been within acceptable limits when produced, showing that they are almost identical with a little variability but within acceptable limits as they are all less than 5% variability.

Shelf-Life

Shelf life can be connected to the stability of the crystal formation in chocolate bars or in protein bars using cocoa butter as the primary fat. Shelf life can also be directly connected to the browning reaction occurring with the sucrose and protein. Both methods are used to determine the shelf life of products that is not a traditional shelf-life study. The titrations had positive values even from high temperatures for 30 days. While the numbers had been low for some values, this could have been a result of the extraction method or using encapsulated vitamin C. the positive values indicate that at 55°C in any of the bars. Under normal temperature conditions, all of the protein bar formulations can be shelf-stable based on the color and retention of vitamin C.

Conclusion

The resulting research indicated that the best combination of whey protein powder to saturated fat ratio is 60 -40% mass/mass. By achieving this innovative ratio, the end product should become 30% protein in a nutrient-dense protein bar. This particular formula of a nutrient-dense protein bar has a content of 30% protein and 36% fat. This particular combination of saturated lipids prevents the fats from leaching out of

the product when temperatures exceed 54°C, even when stored in these conditions for 11 months with no oxidative odor or taste. Cocoa butter, when mixed with palm kernel oil in normal products, causes crystallization, but when whey is added to the mixture, crystal bloom does not occur from competing preferred crystal formation. While in other chocolate bars or chocolate products, this combination of cocoa liquor and palm kernel oil would create a crystal bloom leaving the product unappetizing in appearance and no firm snap.

After the one-month shelf study, no significant difference in the color of the protein bars had been found even at temperatures at 55°C. This also proves true for vitamin C degradation in the protein bars. Based on both of these pieces, the shelf-life study indicated that one month in an accelerated shelf life environment of 55°C replicated one year of shelf stability. The resulting product has an estimated shelf life exceeding 11 years with the proper packaging.

Future Work

Future work to look at how the crystal structure has been formed at a microscopic level would help explain how this particular combination of ingredients allows for all the positive properties of a chocolate bar but without the bloom normally found when mixing two competing lipids. A shelf-life study looking at the breakpoint, lipid oxidation, and water activity could answer questions pertaining to shelf stability and texture over time longer than 30 days. Another project to look at is the primary driving ingredient that allows for the matrix preventing vitamin degradation in this shelf-stable nutrient-dense protein bar. Unconnected to all current work on this project, the ability to increase time

in the day or allow us to go back in time, yes, this was meant to make you smile as science always takes longer than we expect.

Tables and Figures

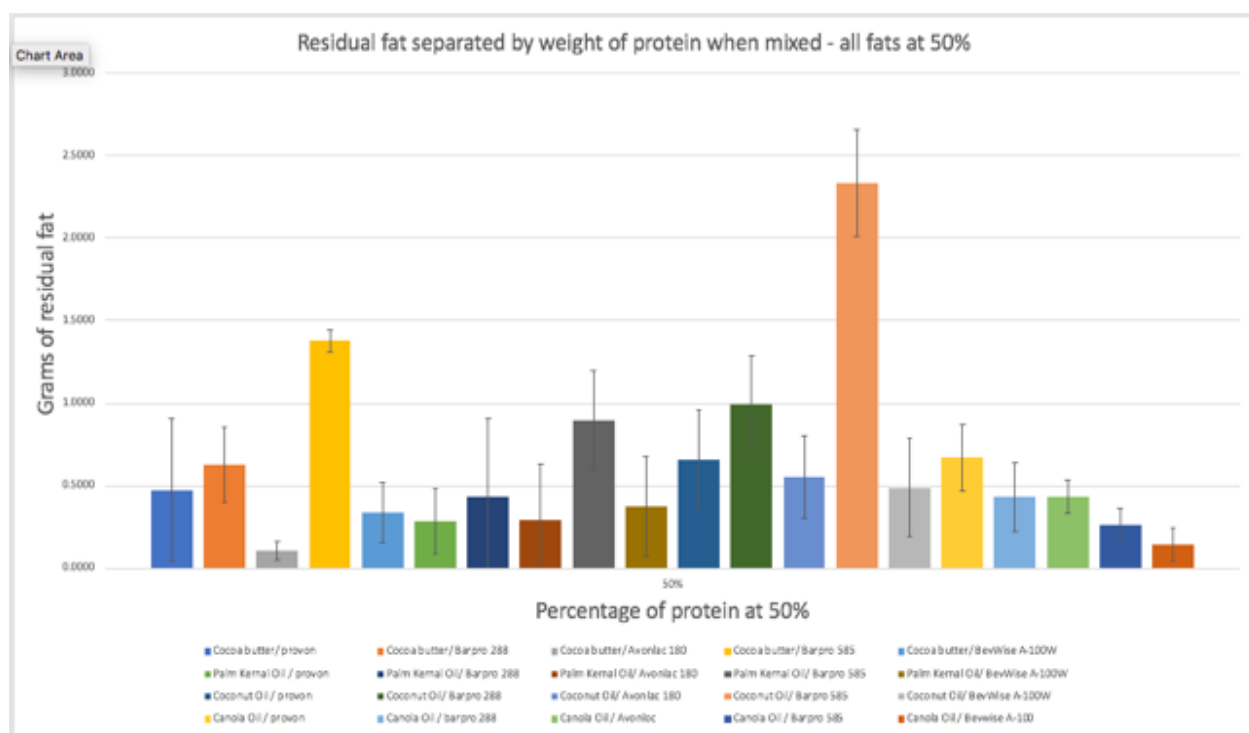


Figure 7 Residual Fat Separated by Whey and Fat Type.

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Appendices: National and International Patent Applications

The national and international patent applications, both entitled “Suspension of Polymerizable Materials in a Solid Fat Matrix to Prevent Aggregation and Extend Shelf Life of Food Materials” is included in the appendices because it provides a view of the project from an intellectual property standpoint.

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(54) Title: SUSPENSION OF POLYMERIZABLE MATERIALS IN A SOLID FAT MATRIX TO PREVENT AGGREGATION AND EXTEND SHELF LIFE OF FOOD MATERIALS

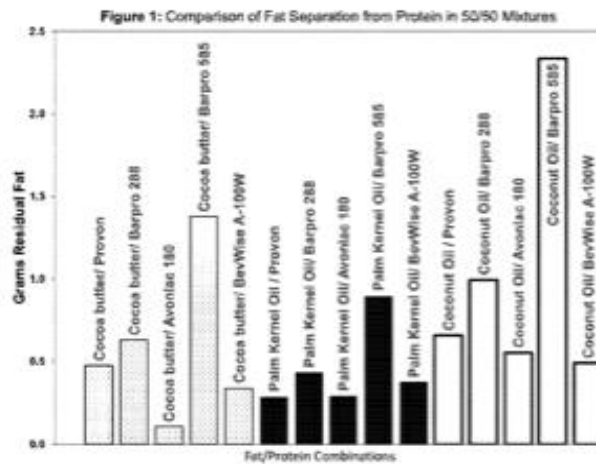


Fig. 1

(57) Abstract: Disclosed is a composition comprising a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further optionally, wherein the dispersion is a uniform dispersion. In some embodiments, the macronutrient is a protein, a carbohydrate, a second type of fat in addition to the one forming the primary fat matrix, water, or a combination thereof. Also disclosed is a process for preparing a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further optionally, wherein the dispersion is a uniform dispersion. In some embodiments, the process comprises: (a) heating the fat matrix to provide a melted or

[Continued on next page]

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heat-plasticized fat matrix; (b) mixing the ingredient into the melted or heat-plasticized fat matrix, optionally wherein the ingredient comprises particles; and (c) cooling the mixture formed in step (b) to form the composition comprising a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further optionally, wherein the dispersion is a uniform dispersion.

DESCRIPTION

SUSPENSION OF POLYMERIZABLE MATERIALS IN A SOLID FAT MATRIX
TO PREVENT AGGREGATION AND EXTEND SHELF LIFE OF FOOD
MATERIALS

5

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application claims benefit of U.S. Provisional Patent Application Serial No. 62/758,310, filed November 9, 2018. The disclosure of this U.S. Provisional Patent Application is incorporated herein by reference in its entirety.

10

GRANT STATEMENT

This invention was made with government support under grant number SP4701-17-P-0049 awarded by the United States Department of Defense's Defense Logistics Agency (DOD/DLA). The government has certain rights in the invention.

15

TECHNICAL FIELD

The presently disclosed subject matter relates in some embodiments to compositions comprising a dispersion of an ingredient, such as but not limited to a macronutrient, micronutrient, dietary bioactive, and/or pharmaceutical ingredient, in a fat matrix. The presently disclosed subject matter relates in some embodiments to a food product comprising a dispersion of a macronutrient in a fat matrix.

20

BACKGROUND

Long chain macronutrients, such as starch and, in particular, protein, tend to self-associate and aggregate over time in food systems. In the case of protein in protein bars, this leads to bar hardening and the end of useful shelf life. The presently disclosed subject matter addresses these and other needs in the art.

25

SUMMARY

This summary lists several embodiments of the presently disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise

30

exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of the presently disclosed subject matter, whether listed in this summary or not. To avoid excessive repetition, this summary does not list or suggest all possible combinations of such features.

Provided in accordance with the presently disclosed subject matter is a composition comprising a dispersion of an ingredient in a fat matrix. In some embodiments, the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further optionally, wherein the dispersion is a uniform dispersion. In some embodiments, the macronutrient is a protein, a carbohydrate, a second type of fat in addition to the one forming the primary fat matrix, water, or a combination thereof.

In some embodiments, the fat matrix comprises a fat that is a solid at temperatures ranging up to 45°C. In some embodiments, the fat matrix comprises a fat that is semi-solid at temperatures ranging up to 40°C.

In some embodiments, the macronutrients are proteins and fats and the proteins and fats are present at ratio of between 1:1 and 1:3 protein to fat by weight. In some embodiments, the macronutrient is a protein of about 18 to 25 kiloDaltons. In some embodiments, the composition has a caloric value ranging from about 4 kilocalories per gram to about 8 kilocalories per gram.

In some embodiments, the ingredient comprises particles. In some embodiments, the ingredient particles have a particle size ranging from about 10 microns to about 200 microns. In some embodiments, the ingredient has a D90 value for particle size of about 100 microns or less.

In some embodiments, the protein comprises predominantly hydrophobic amino acid side chains, as determined by a technique selected from the group consisting of direct sequencing of amino acids, Wimley-White interface or octanol scales, water micro-droplet contact angle measurements, other common measures of peptide and protein hydrophobicity, or any combination of the foregoing. In some embodiments, the protein comprises a whey protein, a casein protein, an egg white protein, an insect protein, a plant protein, or combinations thereof. In some embodiments, the protein comprises at least about 20% of the caloric value of the composition, optionally at least about 25% of the caloric value of the composition.

In some embodiments, the carbohydrate comprises a sugar, a sugar syrup, a polyol, a flour, a starch, a fiber or hydrocolloid, or combinations thereof. In some embodiments, the composition further comprises an ingredient selected from the group consisting of a plasticizer, a thickener, an antioxidant, a chelator, a flavoring, and combinations thereof. In some embodiments, the composition has an average water activity below 0.85, optionally below 0.75.

In some embodiments, the fat is a fat selected from the group comprising cocoa butter, coconut oil, palm kernel oil, tristearin, and fractions and combinations thereof.

In some embodiments, the composition is provided in the form of a bar, a filling, a ganache, a pastry, a shortbread, an icing, a yogurt, a butter, a gel, a paste, a pellet, a sphere (with or without a food-grade candy shell), or an ovoid disc (with or without a food-grade chocolate, candy or gelatin shell/encapsulation).

Provided in accordance with the presently disclosed subject matter is a process for preparing a dispersion of an ingredient in a fat matrix. In some embodiments, the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof. In some embodiments, the dispersion is a uniform dispersion.

In some embodiments, the process comprises (a) heating the fat matrix to provide a melted or heat-plasticized fat matrix; (b) mixing the ingredient into the melted or heat-plasticized fat matrix; and (c) cooling the mixture formed in step (b) to form the composition comprising a dispersion of an ingredient in a fat matrix. In some embodiments, the ingredient comprises particles. In some embodiments, the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof. In some embodiments, the dispersion is a uniform dispersion.

In some embodiments, the fat matrix comprises a fat that is a solid at temperatures ranging up to 45°C. In some embodiments, the fat matrix comprises a fat that is semi-solid at temperatures ranging up to 40°C. In some embodiments, the macronutrients are proteins and fats and the proteins and fats are present at ratio of between 1:1 and 1:3 protein to fat by weight. In some embodiments, the macronutrient is a protein of about 18 to 25 kiloDaltons. In some embodiments, the composition has a caloric value ranging from about 4 kilocalories per gram to about 8 kilocalories per

gram.

In some embodiments, the ingredient comprises particles. In some embodiments, the ingredient particles have a particle size ranging from about 10 microns to about 200 microns. In some embodiments, the ingredient has a D90 value
5 for particle size of about 100 microns or less.

In some embodiments, the protein comprises predominantly hydrophobic amino acid side chains, as determined by a technique selected from the group consisting of direct sequencing of amino acids, Wimley-White interface or octanol scales, water micro-droplet contact angle measurements, other common measures of
10 peptide and protein hydrophobicity, or any combination of the foregoing. In some embodiments, the protein comprises a whey protein, a casein protein, an egg white protein, an insect protein, a plant protein, or combinations thereof. In some embodiments, the protein comprises at least about 20% of the caloric value of the composition, optionally at least about 25% of the caloric value of the composition.

In some embodiments, the carbohydrate comprises a sugar, a sugar syrup, a polyol, a flour, a starch, a fiber or hydrocolloid, or combinations thereof. In some
15 embodiments, the composition further comprises an ingredient selected from the group consisting of a plasticizer, a thickener, an antioxidant, a chelator, a flavoring, and combinations thereof. In some embodiments, the composition has an average water activity below 0.85, optionally below 0.75.
20

In some embodiments, the fat is a fat selected from the group comprising cocoa butter, coconut oil, palm kernel oil, tristearin, and fractions and combinations thereof.

In some embodiments, the composition is provided in the form of a bar, a filling, a ganache, a pastry, a shortbread, an icing, a yogurt, a butter, a gel, a paste, a
25 pellet, a sphere (with or without a food-grade candy shell), or an ovoid disc (with or without a food-grade chocolate, candy or gelatin shell/encapsulation).

In some embodiments, the process comprises holding the melted or heat-plasticized fat matrix for a period of time prior to the mixing. In some embodiments, the holding of the fat matrix is carried out for 1 to 3 minutes. In some embodiments,
30 the mixing the ingredient into the melted or heat-plasticized fat matrix using a low shear mixing method. In some embodiments, the method comprises forming the composition into a bar, a filling, a ganache, a pastry, a shortbread, an icing, a yogurt,

a butter, a paste, a gel, a pellet, a sphere (with or without a food-grade chocolate, candy or gelatin shell/encapsulation), or an ovoid disc (with or without a food-grade chocolate, candy or gelatin shell/encapsulation). In some embodiments, forming the composition comprises forming the composition on a slab roller.

5 Thus, it is an object of the presently disclosed subject matter to provide a composition comprising a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group comprising a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further optionally, wherein the dispersion is a uniform dispersion; and to
10 provide a process for making the composition.

 An object of the presently disclosed subject matter having been stated hereinabove, and which is achieved in whole or in part by the compositions and processes disclosed herein, other objects will become evident as the description proceeds when taken in connection with the accompanying Examples as best
15 described herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

 The presently disclosed subject matter can be better understood by referring to the following figures. The components in the figures are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the presently
20 disclosed subject matter (often schematically). In the figures, like reference numerals designate corresponding parts throughout the different views. A further understanding of the presently disclosed subject matter can be obtained by reference to an embodiment set forth in the illustrations of the accompanying drawings. Although the illustrated embodiment is merely exemplary of systems for carrying out the
25 presently disclosed subject matter, both the organization and method of operation of the presently disclosed subject matter, in general, together with further objectives and advantages thereof, may be more easily understood by reference to the drawings and the following description. The drawings are not intended to limit the scope of this
30 presently disclosed subject matter, which is set forth with particularity in the claims as appended or as subsequently amended, but merely to clarify and exemplify the presently disclosed subject matter.

 For a more complete understanding of the presently disclosed subject matter, reference is now made to the following drawings in which:

Figure 1 is a graph showing a comparison of fat separation from protein in 50/50 mixtures.

DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter, in which some, but not all embodiments of the presently disclosed subject matter are described. Indeed, the presently disclosed subject matter can be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements.

In accordance with aspects of the presently disclosed subject matter, solid fats can be used to uniformly disperse protein particles, reducing the chance that this aggregation will occur and/or preventing this aggregation from occurring. In some embodiments of the presently disclosed subject matter, solid fats are used to disperse protein in general. In some embodiments of the presently disclosed subject matter, and more specifically, the type and particle size of the dispersed proteins, as well as the type and mixtures of solid fats, the processing required to uniformly disperse protein particles in a fat matrix, and the additional ingredients required to prevent fat migration and protein aggregation over the shelf life of a food.

Provided in accordance with some embodiments of the presently disclosed subject matter is a process that enhances the temperature stability and shelf life of complex, high energy density food systems by combining mixtures of particular fats with particular proteins, such as particular whey proteins. The homogenous distribution of protein in a solid fat matrix addresses the phenomena of protein polymerization in high-protein products, which leads to the hardening of food texture and limits shelf life. In some embodiments, the presently disclosed subject matter encompasses a unique 50/50 fat to protein (e.g., dairy protein) mixture that allows for enhanced shelf-stability, reduced protein (e.g., a dairy protein like a whey protein) isolate oxidation reactions, and low water activity. In a particular embodiment comprising this combination, it was shown to be stable at 55°C for over six months in a high energy density protein bar model.

The presently disclosed formulations are adaptable to either sweet or savory products in a variety of forms such as bars, chocolates, and other confectionery products, translating into a new wave of protein-fortified products. The presently

disclosed subject matter are adaptable into other products like candy bars, high-energy density or keto exercise products, or even icings or cake-like products. Some of the savory product flavors can include but are not limited to; bacon, chicken masala, cheese and crackers, beef burgundy, or any flavor desired to infuse into the solid matrix. Evidence of the absence of such a unique combination is readily apparent from the present market offerings.

In a representative embodiment of the presently disclosed formulation, whey protein isolates within a select particle size range (12 μ m-130 μ m), hydrophobicity, and a relatively low shear rate in either a vertical or horizontal mixer are employed. The formulation configured as a bar comprising 30 grams of protein per 100 grams product outperforms what is in the market currently in terms of shelf life and stability to high temperatures. Most other bar products currently on the market consisting of homogeneous mixtures are today are produced using extruder technology. The presently disclosed formulation when configured as a bar can be prepared using a temperature-controlled slab rolling method to distribute the product after low-shear homogenization. The use of fat as a carrier/enrober allows this matrix to prevent oxidation of the protein (e.g., whey protein) that generally occurs after 3 months of exposure to normal room conditions. Protein bars generally are not shelf-stable at high temperatures, 55°C, as most other products or their coatings start to become soft around 40°C and melt at less than 50°C. In standard shelf life testing a product of the presently disclosed subject matter lasts for over six months at 55°C; thus, predicting stability of three years at normal temperature conditions, using both standard and accelerated testing models. One reason for the very long shelf life of the resulting material is due to the low water activity of the presently disclosed formulation, which averages 0.58 Aw.

Thus, aspects of the presently disclosed subject matter include particular fat/protein ratios, such as 50/50 fat/protein ratios; particular combinations of specific fats and whey proteins; particular amino acid compositions in the proteins used; particular protein particle size ranges; the general superiority of whey proteins over mixed casein and whey; the ability of cocoa and palm kernel fats to work together effectively in this system; and the mixing and molding methods used for bar models. Overall, the aspects and qualities of the presently disclosed matrix and the process by

which it is combined provides a novel, adaptable, and non-obvious option to improve the shelf life of high protein products.

DEFINITIONS

The terminology used herein is for the purpose of describing particular
5 embodiments only and is not intended to be limiting of the presently disclosed subject matter.

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

10 All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques or substitutions of equivalent techniques that would be apparent to one
15 skilled in the art. While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

In describing the presently disclosed subject matter, it will be understood that a number of techniques and steps are disclosed. Each of these has individual benefit
20 and each can also be used in conjunction with one or more, or in some cases all, of the other disclosed techniques.

Accordingly, for the sake of clarity, this description will refrain from repeating every possible combination of the individual steps in an unnecessary fashion. Nevertheless, the specification and claims should be read with the understanding that
25 such combinations are entirely within the scope of the invention and the claims.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a component" includes a plurality of such components, and so forth.

30 Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification

and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently disclosed subject matter.

As used herein, the term "about," when referring to a value or to an amount of a composition, mass, weight, temperature, time, volume, concentration, percentage, *etc.*, is meant to encompass variations of in some embodiments $\pm 20\%$, in some
5 embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some
embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as
such variations are appropriate to perform the disclosed methods or employ the
disclosed compositions.

10 The term "comprising", which is synonymous with "including" "containing"
or "characterized by" is inclusive or open-ended and does not exclude additional,
unrecited elements or method steps. "Comprising" is a term of art used in claim
language which means that the named elements are essential, but other elements can
be added and still form a construct within the scope of the claim.

15 As used herein, the phrase "consisting of" excludes any element, step, or
ingredient not specified in the claim. When the phrase "consists of" appears in a
clause of the body of a claim, rather than immediately following the preamble, it limits
only the element set forth in that clause; other elements are not excluded from the
claim as a whole.

20 As used herein, the phrase "consisting essentially of" limits the scope of a
claim to the specified materials or steps, plus those that do not materially affect the
basic and novel characteristic(s) of the claimed subject matter.

25 With respect to the terms "comprising", "consisting of", and "consisting
essentially of", where one of these three terms is used herein, the presently disclosed
and claimed subject matter can include the use of either of the other two terms.

As used herein, the term "and/or" when used in the context of a listing of
entities, refers to the entities being present singly or in combination. Thus, for
example, the phrase "A, B, C, and/or D" includes A, B, C, and D individually, but
also includes any and all combinations and subcombinations of A, B, C and D.

30 As used herein, "dispersion" and "dispersed" refers to distribution of an
ingredient, such as particles comprising an ingredient, within a fat matrix of a
composition. "Homogeneously dispersed," "evenly dispersed," or "uniformly
dispersed" refer to a distribution wherein sedimentation and/or agglomeration are

minimized. For example, in some embodiments, "homogeneously dispersed," "evenly dispersed," or "uniformly dispersed", is generally reflected by a lack of hardening of the composition and extended useful shelf life for the composition. In addition, the term "uniform dispersion" as used herein can refer to a homogenous
5 dispersion of particles having a pre-determined concentration (weight/weight or weight/volume percent).

As used herein "sedimentation" refers to the settling of an ingredient, such as particles comprising an ingredient, typically in an aggregation or agglomeration. As used herein, in some embodiments, an agglomerate or an aggregate is a mass
10 comprising particulate subunits formed via physical (hydrogen bonding, van der Waals, hydrophobic) or electrostatic forces. The resulting structure is called an "agglomerate."

As used herein, "pharmaceutically acceptable" means that the material is suitable for administration to a subject (e.g., a human subject) and will allow desired
15 treatment to be carried out without giving rise to unduly deleterious adverse effects. The severity of the disease and the necessity of the treatment are generally taken into account when determining whether any particular side effect is unduly deleterious.

As used herein, the term "effective" refers to provision of some improvement or benefit to the subject. Alternatively stated, an "effective amount" is an amount that
20 will provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the effects need not be complete or curative, as long as some benefit is provided to the subject. The useful response can provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. The terms also include an amount that will prevent or delay
25 at least one clinical symptom in the subject and/or reduce and/or delay the severity of the onset of a clinical symptom in a subject relative to what would occur in the absence of the methods of the presently disclosed subject matter. Those skilled in the art will appreciate that the useful response need not be complete or curative or prevent permanently, as long as some benefit is provided to the subject.

30 As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, inhibiting the progress of or preventing a disease or disorder. In some embodiments, treatment can be administered after one or more symptoms have developed. In other embodiments, treatment can be administered in

the absence of symptoms. For example, treatment can be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment can also be continued after symptoms have resolved, for example, to prevent or delay their recurrence.

As used herein, the term "low shear rate" refers to a shear rate of less than or equal to 100 rpm or (multiplying by an approximate 1.7 conversion factor) as less than or equal to 170 reciprocal seconds or seconds⁻¹. This definition is based on the optimal rpm values for mixers used to produce bar products. For a reference on rpm vs shear rate, please see Berk Z., Chapter 7, Mixing, In Food Process Engineering. 2013. Food process engineering and technology. Amsterdam, Academic Press, 2013.

COMPOSITIONS

An aspect of the presently disclosed subject matter is that by finely separating out protein particles in a fat medium, proteins are less likely to interact and form hard polymers. Bar hardening is what typically limits shelf life of protein bars. Another aspect of the presently disclosed subject allows for the creation of highly calorie dense foods that will deliver large amounts of calories in small packages. While the presently disclosed subject matter is primarily designed for proteins, it can also function to prevent the association of carbohydrates as well. Dispersing materials with some hydrophobic characteristics into fat can further stabilize the system, as can providing fine particle size dispersion of non-fat components (e.g., protein, sugar, vitamins, and minerals, etc.) in a fat matrix. In some embodiments, fine, uniform dispersion of protein, sugar, vitamin, and other particles in a fat (e.g., solid fat) matrix provides a high calorie, high nutrient density composition capable of withstanding temperatures of 40 degrees Celsius for extended periods of time without deformation or significant loss of flavor and nutritional properties over a two-year shelf-life (as estimated by accelerated storage testing).

In accordance with aspects of the presently disclosed subject matter, provided is a composition comprising an ingredient, such as but not limited to a macronutrient, micronutrient, dietary bioactive, and/or pharmaceutical ingredient, in a fat matrix. In some embodiments, the dispersion is a uniform dispersion. In some embodiments, the macronutrient is a protein, a carbohydrate, a second type of fat in addition to the one forming the primary fat matrix, water, or a combination thereof. In some

embodiments, the composition comprises a dispersion of protein and other macronutrients or micronutrients into a solid fat matrix that maximizes calorie density, flavor, and shelf stability. In some embodiments, a food product composition comprising a bar is more calorie-dense than other bars currently available in the art.

5 In some embodiments, the composition has a caloric value ranging from about 4 kilocalories per gram to about 8 kilocalories per gram. By way of elaboration and not limitation, 4 kilocalories per gram can be achieved through inclusion of a protein or a carbohydrate as the only macronutrient. Additionally, 8 kilocalories can be achieved as a weighted average of 1:3 protein to fat.

10 In accordance with aspects of the presently disclosed subject matter, approaches are provided for creating an anaerobic environment. An anaerobic environment has risks and benefits. A risk is *Clostridium botulinum* (*C. bot*) outgrowth, which can be avoided via water activity control as described herein below. A benefit is that nutrients, like fat, protein, and vitamins that can oxidize, will be
15 prevented from doing so by a low or no oxygen environment. Thus, in some embodiments, approaches in accordance with the presently disclosed subject matter comprise dispersing nutrients in a fat matrix that does not result in the outgrowth of *C. bot* spores or other anaerobes of concern. The United States Food and Drug Administration (FDA) indicates that *C. bot* does not produce toxin at water activities
20 below 0.85. In some embodiments, shown herein below in the Examples, a food product composition in accordance with the presently disclosed subject matter has an average water activity of 0.51 and 0.65 for a chocolate bar and a cheese bar, respectively. In some embodiments, a composition in accordance with the presently disclosed subject matter has an average water activity below 0.85, optionally below
25 0.75, further optionally below 0.70, further optionally below 0.65, further optionally below 0.60, further optionally below 0.55.

In some embodiments, certain micronutrients and/or dietary bioactive ingredients, for example, vitamins and minerals, are suspended in the solid fat matrix in order to prevent lipid oxidation and/or flavor changes over time. Given the high
30 fat nature of the product, steps can be taken to prevent contact of transition metals, photosensitizing vitamins such as riboflavin, and other initiators and promoters of lipid oxidation. This is accomplished, for example, via the use of established nutrient encapsulation technologies.

In some embodiments, the presently disclosed subject matter provides a high calorie, high protein bars that will not harden and, therefore have extended shelf lives. In some embodiments, the bar has a caloric value ranging from about 4 kilocalories per gram to about 8 kilocalories per gram.

5 In some embodiments, the presently disclosed subject matter provides lower-calorie confections. To elaborate, fat has 9 kcal/g and protein has 4 kcal/g. This means that adding non-fat materials effectively dilutes the calorie content of high fat products.

10 In some embodiments, a composition of the presently disclosed subject matter comprises 25 to 35 grams protein per 100 grams of material, such as 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 grams of protein per 100 grams material.

In some embodiments, the fat matrix comprises a fat that is a solid at temperature ranging up to 45°C, including solid at 40°C, 41°C, 42°C, 43°C, 44°C, and 45°C. In some embodiments, the fat matrix comprises a fat that is semi-solid at 15 temperatures ranging up to 40°C, including semi-solid at 35°C, 36°C, 37°C, 38°C, 39°C, and 40°C. Cocoa butter, coconut oil, palm kernel oil, tristearin, and fractions and combinations thereof, are representative fats used to achieve a high-melting bar. Other lower melting fats, or semi-solid fats and fat mixtures (e.g., mono-unsaturated 20 fats such as triolein mixed with cocoa butter, coconut oil, palm kernel oil, or tristearin), are employed for the creation of pastes. Fully hydrogenated, zero trans fats (e.g., fully hydrogenated soybean, coconut, or palm kernel oil) are also used to provide high-melting properties. In embodiments comprising chocolate, a chocolate liquor can be added, which provides additional fat content. In some embodiments, the matrix comprises a mixture of fats, such as a mixture of cocoa butter and palm kernel oil. 25 In some embodiments, the mixture of cocoa butter and palm kernel oil comprises 10% by weight palm kernel oil. In some embodiments, palm kernel oil can range between 5% and 50% by weight (5/95 to 50/50 ratio) with cocoa butter, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50% palm kernel oil.

30 The fat matrix is hydrophobic. Proteins can vary in hydrophobicity/hydrophilicity. In some embodiments using dairy protein (for example, whey protein), the protein comprises high aspartic acid (8.0-9.5 mg/100 g), threonine (5.0-6.5 mg/100 g), and low tyrosine (2.0-3.0 mg/100 g) levels relative to other dairy proteins. In some embodiments, a more hydrophobic protein fraction

improves the stability of the suspension. Thus, in some embodiments, the protein comprises predominantly hydrophobic amino acid side chains, as determined by direct sequencing of amino acids, by the Wimley-White interface or octanol scales, by water micro-droplet contact angle measurements, and/or by other common measures of peptide and protein hydrophobicity.

In some embodiments, the protein comprises a whey protein, a casein protein, an egg white protein, an insect protein, a plant protein, or combinations thereof. In some embodiments, the plant protein is Maringa. In some embodiments, molecular weights for the protein, such as but not limited to whey proteins, vary from 18 kilodaltons (kD) to 25kD, including 19 kD, 20 kD, 21 kD, 22 kD, 23 kD, 24kD, and 25kD. In some embodiments, the protein comprises at least about 20% of the caloric value of the composition, optionally at least about 21% of the caloric value of the composition, optionally at least about 22% of the caloric value of the composition, optionally at least about 23% of the caloric value of the composition, optionally at least about 24% of the caloric value of the composition, optionally at least about 25% of the caloric value of the composition.

In some embodiments, wherein the macronutrients are proteins and fats, the proteins and fats are present at ratio of between 1:1 and 1:3 protein to fat by weight, including any ratio combination with this range, e.g. 1:1.2, 1:1.5, 1:1.7, 1:2, 1:2.3, 1:2.6, 1:2.9, 1:3 and the like. In some embodiments, the fat predominates. In some embodiments, the protein is dispersed in fat at an approximately 1:1 ratio on a weight/weight basis, i.e., 50% protein/50% fat by weight.

In some embodiment, when the ingredient comprises a protein, the protein ingredient comprises has a pH ranging between 6.5 and 3.5, including pH 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, and 3.5 when hydrated. The pH of the hydrated protein is indicative of the process used to produce it. By way of example and not limitation, the proteins used in the studies described herein below reflect the pH of normal bovine milk (~6.5) or proteins at pH ranges below that of bovine milk. In some cases, lower pH could be a result of microbial fermentation related to cheese production or to direct addition of acid to lower pH. In some embodiments of a composition of the presently disclosed subject matter comprises a calcium level below 900 mg per 100 grams protein. Furthermore, in some embodiments when the ingredient is a protein, the sugar content of the protein source comprises less than about 2% by weight, such as below about

1.79% by weight. To elaborate, in some embodiments, low sugar (such as lactose) in a dairy protein would lessen browning reactions that could limit shelf life. Furthermore, in some embodiments when the ingredient is a protein, the water content of the protein source comprises less than about 6% by weight, such as below about 6,
5 5, 4, 3, or 2% by weight. By way of further exemplification and not limitation, a dry protein powder may more easily and fully mix with pure fat which, by definition, has a very low water content.

In some embodiments, the composition comprises a carbohydrate. In some embodiments, the carbohydrate comprises a sugar, a sugar syrup, a polyol, a flour, a
10 starch, a fiber or hydrocolloid, or combinations thereof. In some embodiments, the carbohydrate comprises at least about 40% of the caloric value of the composition, optionally at least about 45% of the caloric value of the composition. In some embodiments, the carbohydrate is dispersed in fat at a ratio varying from 1:1 to 2:1 on a carbohydrate to fat weight/weight basis, e.g. 1:1, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1,
15 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, and the like.

In accordance with aspects of the presently disclosed subject matter, approaches for maintaining fine particle dispersions and keeping the particles suspended until the solid fat hardens are provided, as are approaches for accomplishing this on a large scale. Particle size of dispersed materials and
20 heating/cooling of fat are aspects of these approaches.

In some embodiments of the presently disclosed compositions, fats are the primary dispersing agent. In some embodiments, the dispersion is facilitated by inclusion of proteins with a high degree of hydrophobicity and a fine particle size, as mentioned elsewhere herein. Additionally, mixing of proteins with other dry
25 ingredients in a particular order can facilitate the maintenance of a uniform dispersion in the fat matrix. By way of non-limiting example, sugar, starches and proteins can be mixed together first in order to disperse micronutrients and other dry ingredients present at low concentrations. Alternatively, as another non-limiting example, protein can first be mixed with liquid or partly melted fat in order to encourage maximum
30 hydrophobic interactions. The pH of the protein used may first be adjusted to a point close the pI (isoelectric point) in order to minimize charge and maximize hydrophobic interactions. The other dry ingredients would then be added in. In some embodiments, other ingredients (e.g., a natural plasticizer like glycerin) are employed.

In some embodiments, a composition in accordance with the presently disclosed subject matter comprises an ingredient, such as but not limited to a macronutrient, a micronutrient, a dietary bioactive, and/or a pharmaceutical ingredient, having a particle size is that does not exceed a size that can be perceived as unacceptable in texture or mouthfeel by human consumption. In some
5 embodiments, the ingredient has a particle size that enables the material to be considered an acceptable direct-addition food ingredient. In some embodiments, the ingredient has a particle size ranging from about 10 microns to about 200 microns, including any value within this range, such as but not limited to 12 microns, 25
10 microns, 50 microns, 75 microns, 100 microns, 125 microns, 130 microns, 150 microns, 175 microns, 180 microns and 200 microns.

In some embodiments, the ingredient has a D90 value for particle size of about 100 microns or less. Particle size production techniques, such as but not limited to food-based particle size production techniques, do not necessarily produce uniformity
15 of size, but rather maintains a typical bell-curve distribution. Thus, particle sizes are typically defined by D-values, where D-90 equates to 90% of particle sizes falling below stated value. D-50 and/or D-10 are often cited as reference points along with D-90. In some embodiments, the particle size criteria to enable the ingredient to be considered an acceptable direct-addition food ingredient comprise a D90 value for
20 particle size of about 100 microns or less, in some embodiments about 95 microns or less, in some embodiments about 90 microns or less, in some embodiments about 85 microns or less, in some embodiments about 80 microns or less, and in some embodiments, about 75 microns or less. In some embodiments, the ingredient has a D50 value for particle size of about 50 microns or less, in some embodiments 25
25 microns or less, in some embodiments, 10 microns or less.

Representative particle size goals for ingredients in accordance with the presently disclosed subject matter include:

D-90 = 100 micron, 95 micron, 90 micron, 85 micron, 80 micron, or 75 micron.

30 D-50 = 50 micron, 25 micron, or 10 micron.

D-10 = inconsequential.

Dietary bioactive applications and/or pharmaceutical applications (e.g., prebiotics, probiotics, antioxidants, compounding of various medications) can include

blending active ingredients in a fat matrix to slow their release, especially for pharmaceuticals that "should be taken with food." This could also extend to dietary bioactive products that are being created in the market in edibles (e.g. cannabis-based products). In some embodiments, an effective amount of a dietary bioactive ingredient and/or pharmaceutical ingredient is included in the composition.

In some embodiments, the composition comprises an ingredient selected from the group comprising a plasticizer, an emulsifier, a thickener, an antioxidant, a chelator, a flavoring, caffeine, theobromine, and combinations thereof. Representative plasticizers include but are not limited to glycerin. Representative emulsifiers include, but are not limited to, lecithin and other phospholipids, mono and diglycerides, tweens and spans. Representative thickeners include but are not limited to gums (e.g., xanthan gum and guar gum), hydrocolloids, and syrups. Representative antioxidants include but are not limited to vitamin E and BHT. Representative chelators include, but are not limited to EDTA and citric acid. Use levels for the above ingredients can vary between 0.01% to 5%, depending on the ingredient in question.

Flavoring ingredients can also be included in the presently disclosed compositions. Desirable flavors include both sweet (e.g., chocolate) and savory (e.g. cheese) flavors, with and without caffeine. Additional optional characteristics include maintenance of flavor and nutrition, as well as a lack of bar hardening, melting, and discoloration over an approximately two-year shelf life. Representative flavorings are disclosed in the Examples, set forth herein below. Sweet and savory flavors are provided, such as but not limited to chocolate, cheese and crackers, cheddar bacon and jalapeno bacon. (Use levels would vary based on flavors from the 1-200 parts per million level for pure flavor active compounds to the 1-10% (by weight or by volume) level for food-based flavors, such as cheese powder.

The presently disclosed compositions can be used in accordance with a variety of applications as would be apparent to one of ordinary skill in the art upon a review of the instant disclosure. Representative applications included but are not limited to shelf-life extension; delivery and/or protection of protein, vitamins, and other labile nutrients; flavor protection; probiotic delivery vehicle; and/or fiber delivery vehicle.

In some embodiments, the composition is provided in the form of a bar, a filling, a ganache, a pastry, a shortbread, an icing, a yogurt, a butter, a paste, a gel, a

pellet, a sphere, or an ovoid disc. Thus, in addition to bars, provided are spherical or ovoid candies, which can be chocolate, candy or gelatin-coated, but do not have to be chocolate, candy or gelatin-coated. Such candies can comprise a similar protein/fat composition as a bar as disclosed herein, but are of smaller size than bars in order to
5 allow for an alternate approach for delivering a very similar nutrient profile or foods that are bite size for quick delivery in activities like rock climbing when light, portable products are desired.

Representative applications thus include M&M-like or round/ovoid small serving size products with a similar nutritional profile as bar embodiments; highly
10 fortified nut butters using more plastic or semi-solid fats; cheese fillings for crackers; sweet fillings for cookies; icing; ganache or any confections requiring high fat content; pastries; shortbreads; Ice cream inclusions; cheese products (cream cheese, processed cheese sauce product); thickened yogurts; modified butters; long-term nutrient storage without refrigeration; high protein "edibles" that contain CBD or
15 other active ingredients in markets where their sale is permitted; and the dispersion of pharmaceuticals best consumed with food. The presently disclosed compositions thus shift protein bar, sports nutrition, pharmaceutical, and "edibles" design and could utilize modified chocolate/confection processing lines for, as well as design of high fat- or oil-based food products that people consume.

20 PROCESSES FOR MAKING COMPOSITIONS

The presently disclosed subject matter provides processes to make the above-described compositions to uniformly disperse an ingredient (e.g. proteins/carbohydrates/etc.) in a fat matrix. In some embodiments, disclosed is a process for preparing a composition comprising a dispersion of an ingredient, such as
25 but not limited to a macronutrient, micronutrient, dietary bioactive, and/or pharmaceutical ingredient, in a fat matrix. In some embodiments, the process comprises heating the fat matrix to provide a melted or heat-plasticized fat matrix; mixing the ingredient into the melted or heat-plasticized fat matrix; and cooling the mixture to form the composition comprising a dispersion of the ingredient, such as
30 but not limited to a dispersion of a macronutrient, micronutrient, dietary bioactive, and/or pharmaceutical ingredient, in a fat matrix. Other ingredients can also be mixed into the fat matrix, including but not limited to a plasticizer, a thickener, an antioxidant, a chelator, a flavoring, and combinations thereof. In some embodiments,

the dispersion is a uniform dispersion. The heating can be done to any desired temperature to melt the fat matrix, such as based on the fat matrix components chosen, as would be apparent to one of ordinary skill in the art upon a review of the instant disclosure. By way of example and not limitation, the heating can be done at about
5 50°C. In some embodiments, the heating and/or cooling comprises tempering the mixture; however, tempering is not required.

In some embodiments, the construction of the matrix begins with melted fat incubated for a predetermined period of time, such as about 1-3 minutes. In some embodiments, the melted fat is also held at a predetermined temperature, such as but
10 not limited to about 25°C. By way of exemplification and not limitation, after the fat melts, it is held for one minute at 25°C. Including a hold time is an aspect of a process of the presently disclosed subject matter, as processes in the are typically designed to quickly move to bar ingredient mixing and bar molding steps before fats re-solidify. In some embodiments, once fat has been held for a desired hold time, such as one
15 minute, all the dry ingredients are added and the product is mixed with a low shear rate, e.g., less than 100 rpm or a shear rate of less than 170 seconds⁻¹. Monitoring shear rate is also an aspect of some embodiments of the presently disclosed subject matter. That is, it can be the case that if too much shear is applied in the mixing process, the matrix does not form correctly. Thus, the method further comprises using
20 a low shear mixing method, such as in either a vertical or horizontal mixer. The composition is then formed using a slab roller, such as a heated slab roller. In some embodiments, an extrusion method is not employed.

In some embodiments, the method comprises forming the composition into a desired shape or format, such as but not limited to a bar, a filling, a ganache, an icing,
25 a yogurt, a butter, a paste, a gel, a pellet, a sphere (with or without a food-grade candy shell), or an ovoid disc (with or without a food-grade candy shell). In some embodiments, forming the composition comprises layering the composition into a mold or form of a desired shape. The method further comprises using a low shear mixing method, such as in either a vertical or horizontal mixer. The composition is
30 then formed using a slab roller, such as a heated slab roller. In some embodiments, an extrusion method is not employed.

In some embodiments, the presently disclosed subject matter provides approaches to more homogenously mix fat and protein together and to examine the effects of proteins with differing degrees of hydrophobicity on bar prototypes.

The materials and equipment employed include basic industrial kitchen (hot plate, mixing bowls, sauce pot, scale, slab roller) and chocolate tempering machine
5 for compositions that include chocolate. However, tempering is not required. In some embodiments, the presently disclosed processes employ modified chocolate/confection processing lines for manufacture. In the case of the preparation of spherical or ovoid candies, which can be candy coated, a form of the desired size
10 is used and then the formed composition is placed in a standard tumbler for candy coating. In the case of compositions comprising chocolate, a conching step can be performed to enhance smoothness.

Obtaining and maintaining a fine dispersion of particles may be achieved in a number of ways, including but not limited to the use of various types of vertical and
15 horizontal tumblers and mixers, with or without scraped surface. Rotation, vibration, ultra-sound, or electric current (in the case of charged particles) may be used to maintain a fine particle dispersion until the mixture solidifies, gels, freezes, or is otherwise stabilized.

20 EXAMPLES

The following examples are included to further illustrate various embodiments of the presently disclosed subject matter. However, those of ordinary skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result
25 without departing from the spirit and scope of the presently disclosed subject matter.

The examples include both chocolate and cheese flavors in caffeinated and non-caffeinated varieties. In one example a more savory bar composition was prepared. A cheese or "cheese and crackers" flavor was viewed as the best approach. Glycerin and a potato starch slurry were used to adjust texture and water activity in
30 the cheese flavor.

Table 1. Chocolate Bar Prototype Nutrient Profile and Ingredients

Nutrient/Property	Value	Percent of Calories
Total Calories	723.5	N/A
Protein	34 grams	19%
Carbohydrates	62.5 grams	35%
Fat	37.5 grams	47%
Fiber	1.2 grams	N/A
Bar Weight	134 grams	N/A
Water Activity	0.50	N/A
Melting Point	>40°C	N/A

Ingredients (listed in order by weight): Cocoa liquor, Whey protein Isolate, Oat Flour, Invert sugar, Cocoa Butter, Palm Kernel Oil, Cocoa Powder, Salt, Caffeine.

5

Table 2. Savory Bar Prototype Nutrient Profile and Ingredients

Nutrient/Property	Value	Percent of Calories
Total Calories	710	N/A
Protein	35 grams	20%
Carbohydrates	66 grams	37%
Fat	34 grams (zero trans)	43%
Fiber	1.2 grams	N/A
Bar Weight	140 grams/4.9 ounces	N/A
Water Activity	0.65	N/A
Melting Point	>40°C	N/A

Ingredients (listed in order by weight): Whey protein Isolate, Oat Flour, Cheddar Cheese Powder, Invert Sugar, Cocoa Powder, Palm Kernel Oil, Potato Starch, White Cheese Powder, Corn Syrup, Glycerin.

10

USE OF REDUCING SUGARS IN CHEESE BARS

The use of reducing sugars, especially in cheese bars, caused non-enzymatic browning reactions to occur when exposed to high temperatures (40 degrees Celsius) or to weeks of storage at room temperature. This might be partly due to packaging.

5

MODEL PROTEIN BAR SYSTEM

Fat/protein combinations and processes were tested both in a nutrient-dense protein bar model system and in an isolated fat/protein model. Referring to the protein bar model first, the reason for testing fat/protein combinations in a bar model was to provide an extended shelf-life protein bar for the military. Currently in the protein bar market, protein bars fall into two general categories: extruded bars, with or without layers, and multi-component bars held together with sugar syrups. Extruded bar layers may or may not contain inclusions and the entire bar is often enrobed in a chocolate-like coating. A popular example of the extruded, enrobed bar approach would be PowerBar® products. The extruded bar coatings melt at around 34°C and the extruded center hardens over six months to a year of storage. Examples of the “glued together” approach would include Clif Bars® and Friend Bars®. These are mixtures of ingredients “glued” together with a sugar syrup. These “glued” type bars typically contain significant quantities of unsaturated fat, resulting in short shelf lives due to rancidity.

20

In contrast, the presently disclosed bars use a solid fat matrix to suspend homogenously dispersed particles, in a way that is analogous to chocolate (in terms of suspending solid particles in fat). Although there is an analogy to chocolate, the model bars are quite distinct from chocolate in terms of composition and processing, such as but not limited to with regard to their high protein content, relatively low sugar content, fat combinations, and lack of tempering. The resulting model protein bar has higher melting temperature and longer shelf life than bars currently on the market, due to particular ingredient mixtures and processing.

25

In some embodiments, the ingredient matrix disclosed herein encapsulates protein within solid fat, allowing the two macronutrient components to become bound together in a way that prevents separation. In a particular, non-limiting example, the model bar-matrix uses three key ingredients: whey protein isolate, cocoa butter, and palm kernel oil. Choosing a mixture of cocoa butter and palm kernel oil (PKO) was

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not originally planned as these fats do not generally work well together. In some embodiments, pure cocoa butter is used due to its shelf stability and high melting temperature. But, after finding that even higher melting temperatures were needed, mixtures of cocoa butter and PKO were tested. When cocoa butter and PKO are mixed, they typically "bloom", meaning that the mixed fats produce large crystals that can move to the surface of the food, disrupting appearance and texture. Cocoa butter and cocoa products' ideal crystalline structure, termed "beta", has a melting point of 34-35°C and is stable until that point. (Minifie 1999). Conversely, PKO assimilates into beta-prime crystals. When PKO is mixed with cocoa butter products, it typically becomes the dominating crystal structure and seeds the cocoa butter with a less desirable (grainy) beta prime crystalline structure. This is why chocolate companies do not mix PKO with cocoa butter for the production of chocolate bars. Pure PKO can, however, be used as a cocoa butter replacement also known as Cocoa Butter Equivalent (CBE). Thus, an aspect of the presently disclosed subject matter is the successful combination of cocoa butter and PKO without fat bloom in a bar system.

In an exemplary bar model system, six core ingredients were employed: whey protein, cocoa liquor (a natural mixture of 50% cocoa solids and 50% cocoa butter), sucrose syrup (a mixture of water and sucrose), oat flour, cocoa powder, and salt. These ingredients were combined to create a slab-rolled bar product. The process of making the model system includes the solid fats being melted at or above 50°C. After the fat melts, it is held for one minute at 25°C. Including a hold time is an aspect of a process of the presently disclosed subject matter, as processes in the art are typically designed to quickly move to bar ingredient mixing and bar molding steps before fats re-solidify. Once fat has been held for a desired hold time, such as one minute, all the dry ingredients are added and the product is mixed using a pastel style mixing process with a low shear rate, e.g., less than 100 rpm or a shear rate of less than 170 seconds⁻¹. Monitoring shear rate is also an aspect of some embodiments of the presently disclosed subject matter. That is, it can be the case that if too much shear is applied in the mixing process, the matrix does not form correctly. This style of mixing is also in contrast to typical processes in the art, which aim for a fast, high rate of mixing to create the product quickly. A horizontal mixer can also be used at low speed to allow the product to mix in relatively low shear rate conditions. When mixing of the product is complete, the matrix is clay-like and malleable enough to form any shape.

The next step is to load the mixture into a hopper for metering onto a slab-rolling surface. This is in contrast to an extrusion process. It was observed that if the product is extruded while warm and malleable, the pressure of extrusion forces the bonding between the protein (e.g., whey protein) and the fat to collapse, causing the product to lose the fat from the matrix. If extrusion continues, the loss of fat from the matrix will eventually cause mechanical failure of the extruder as the product cools. This result is surprising, as most many bar producers use extruders instead of rolling.

The model bars of the presently disclosed subject matter have been subjected to extensive shelf life testing, both accelerated testing (one year at 55°C) and two years at 25°C without significant declines in sensory quality.

Over the course of model bar testing, PKO was introduced to the original set of six ingredients and a variety of flavors were produced (peanut butter, cheese, caramel, etc.) During model bar testing it became apparent that the most stable fat to protein ratio was 50/50 weight/weight, regardless of changes made to other ingredients. The mixture of 50/50 fat/protein can be adopted to a large number of products. Any product that can be produced using a high saturated fat mixture such as ganaches, pastries, shortbreads, or other food systems. The presently disclosed subject matter can also be used to introduce a significant source of protein into these and other food systems. By changing to other fat sources for the formation of the matrix of fat and protein or substituting part of the fat component, one can achieve a higher protein product without a dramatic change in flavor or texture. For example, a croissant might be constructed substituting one-third of the butter with the matrix of 50% cocoa butter and 50% whey protein. The newly constructed croissant-like pastry will contain whey protein that can be used in place of adding eggs or meat-based products for a breakfast sandwich. Another example might be to take the ganache from inside of a chocolate truffle and replace some of the cocoa liquor with this matrix adding more protein while not disturbing the texture or flavor. Thus, the presently disclosed subject matter provides for unexpected new products, as it is believed that one of ordinary skill in the art would not think about replacing saturated fat with a fat/protein matrix to create a healthy high protein product.

ANALYSES OF DAIRY PROTEINS USED IN MODEL SYSTEMS

The observation that cocoa butter and PKO combinations, mixed with 50/50 weight/weight ratio, produced highly stable model protein bars led to closer analysis of the specific types of fat and protein that could be used to optimize stability. While cocoa butter and PKO are relatively homogenous ingredients with known compositions, the dairy protein ingredients used were much more variable. The protein powders used were (all commercially available from Glanbia plc, Kilkenney, Ireland): Barpro 585, a milk protein isolate (*contains both casein and whey proteins*), Barpro 288, a dairy protein blend (*contains both casein and whey proteins*), Avonlac 180, a concentrated whey protein, Provon 190, a whey protein isolate, and Bevwise A-100W, a whey protein isolate. In order to better characterize the fat/protein matrix, moisture and particle size analyses were conducted on the protein powders used. Moisture was measured using the Official Methods of Analysis, (AOAC International) Method 927.05. and is reported in **Table 3**, along with percent protein, sugar, and calcium. Protein particle size distribution was determined using a Microtrac Sympatec (Microtrac Inc., Montgomeryville, Pennsylvania, United States of America) particle size analyzer and a Gilson (Gilson Co., Inc., Lewis Center, Ohio, United States of America) sieve shaker, Model SS-15 as per AOAC 973.03. Protein particle size ranged from 12 μm and 180 μm , with the bulk in the range of 50 μm to 120 μm . Particle size varied between the isolate type. For Barpro 288, the range is described as having a mean volume (MV) of 70.926 μm with a standard deviation (SD) of 48.67 μm . When looking at Avonlac 180, it has an MV of 21.416 μm and SD of 26.99 μm . Next, Barpro 585 has a MV of 63.18 μm with SD of 34.43 μm . Proven 190 has an MV of 48.37 μm with an SD of 25.54 μm .

Table 3. Moisture, Protein, Sugar and Calcium Contents in Proteins Tested

Protein	% Moisture	% Protein	% Sugar	mg Calcium *
Barpro 288	4.14	90.36	1.79	945.20
Barpro 585	5.29	86.14	4.0	2,100.00
Avonlac 180	4.00	80.79	1.7	880.00
Provon 190	3.61	94.02	2.0	402.00
BevWise A100W	2.36	84.10	0.7	480.00

*mg calcium reported on a per 100 gram whey protein basis.

Whey protein compositions were analyzed via acid-hydrolysis to liberate amino acids, followed by UPLC analysis, as per AOAC method 982.30. Cystine and methionine were calculated using AOAC Method 982.30. Tryptophan analysis was conducted using AOAC Method 988.15. Amino acid profiles among proteins tested were similar, differing primarily in the relative amounts of aspartic acid, threonine, and tyrosine, as shown in **Table 4**. Lowest values are in bold.

Table 4. Amino Acid Content Of Various in Proteins Tested

Protein Powders	Aspartic Acid mg/100g	Threonine mg/100g	Tyrosine mg/100g
Barpro 288	9.1	5.6	3.5
Barpro 585	5.9	3.3	4.3
Avonlac 180	8.7	5.3	2.2
Provon 190	9.9	6.1	2.6
BevWise A100W	8.8	5.9	2.4

ISOLATED FAT/PROTEIN MIXTURES

A rapid method was developed to determine the stability of specific fat/protein ratios outside of a food matrix. Briefly, 10 grams of solid fat was melted, mixed with varying weights of dairy proteins (0.0, 2.5, 5.0, 7.0, and 10 grams). The mixtures were re-solidified to create weight/weight fat/protein ratios of 100/0, 80/20, 70/30, 60/40, and 50/50. These fat/protein mixtures were placed onto a filter in the upper portion of 50 ml centrifugation tubes. The fat/protein mixtures were heated to 3°C above the melting point of the fat and centrifuged in order to determine how much fat would separate from each fat/protein mixture. The best fat/protein ratio in terms of fat retention was found to be 50/50, which reflects what was observed in the bar model. **Figure 1** shows the differences in fat loss for varying fat/protein combinations at weight/weight ratio of 50/50. Three proteins, all proteins comprising whey, provided the highest degree of stability (least fat loss) across all fats tested: Provon 190, Avonlac 180, BevWise A-100w.

DISCUSSION OF EXAMPLES

Commercially available protein powders have a wide range of functional properties and can be used in a variety of foods and beverages. Due to the dynamic nature of protein interactions within food matrices, product developers must be careful in the selection of protein powder based on the desired interactions needed in their system. For the presently disclosed subject matter, it was desired to elucidate the properties needed in a protein for the creation of a stable fat/protein matrix. We determined that a desirable particle size for fat/protein interactions is between 11 μm and 130 μm , based on the superior performance of the three whey proteins. These factors place Avalac 180, Provon 190, and Bevwise A-100w, inside the range of protein with particle sizes needed to develop ideal mixtures. Barpro 288 and 585 had mean volumes of 70 μm and 63 μm respectively at the upper end of the desired particle size range. However, considering the variability around the mean volume, SD of 48.67 μm and 34.43 μm respectively indicates that these protein powders tend to have particles that range higher than that of the desired protein systems. While it is not desired to be bound by any particular theory of operation and considering the underlying cause for the ideal particle size range, it is postulated that smaller particles should be absorbed into the saturated fats more efficiently allowing for better binding and a potentially smoother product. It was also observed that a mixture comprising greater than 60% protein to 40% fat were very grainy and powdery. While it is not desired to be bound by any particular theory of operation, this larger amount of protein tends to encourage protein to protein interactions, resulting in clumping of whey protein within the fat mixture. The protein and fat matrix can be adjusted to meet the requirements for many different products, not just protein bars.

The moisture content of the protein (e.g., whey protein) is desirably at a low level for our fat containing matrix. While it is not desired to be bound by any particular theory of operation, it is believed that the low moisture protein powders are better able to associate with the lipophilic and hydrophobic fats. The data provided herein supports a connection between the moisture content of the whey protein powder and its solubility into fats. With this idea in mind, it is desired in some embodiments that the moisture content for a stable matrix needs to be below 4%. While it is not desired to be bound by any particular theory of operation, it is believed that the low moisture ensures that the hydrophobicity of the powder is not repressed and allows for the

absorption of protein into the melted lipid mixture. Both of the Barpro products have a moisture content above 4% (Barpro 288 at 4.14% and Barpro 585 at 5.25). Thus, the analysis presented herein predicts that these higher moisture values will inhibit stable matrix formation. The presently disclosed novel analytical method proved useful for testing the ability of various commercially available proteins and fats to form a stable matrix. Of the five protein powders tested, the whey protein isolates performed better in forming a stable matrix. While the other powders contained some whey, they also contained other proteins. In addition to the difference in protein composition between whey protein isolate (WPI) and the other powders there are significant processing differences in the production of these powders. The process of producing an isolate is vastly different from making a concentrate or dairy blend. For example, the WPI is a result of cheese making while the concentrate is made from separating the whey from the milk in an ultrafiltration step. As for the milk protein isolate, the isolation process seeks to partially remove non-protein components, from low-fat milk with the aim of producing a mixture of casein and whey protein product. The difference between the three different processes briefly described above shows that the methods produce a different complement of proteins should be considered in selecting a protein for a protein fat matrix.

Furthermore, the sugar content of the sampled proteins varies significantly from 0.7 to 4.0 grams of sugar per 100 grams protein powder. This is an extensive range, and adds to the noted differences between the proteins produced by different separation or recovery processes. A trend was observed in the association of protein to fat such that when the sugar content for a protein powder was below 1.7% sugar the ability to bind with desirable fats increases. Those powders with sugar content below 1.7% are Avonlac 180, Provon 190, and BevWise A-100w. The test samples with sugar contents above 1.7% were the two Barpro products. Thus, sugar content can also be considered in preparing a protein fat matrix.

The calcium content of the protein powders also correlates with the overall ability of the protein to bind to the fat matrices. Higher calcium contents are correlated with less than desired matrix formation. Levels of calcium lower than 900 mg of calcium per 100 grams of protein are found in powders possessing the ability to form stable protein fat interactions. The products that fall into the ideal range include: Avonlac 180, Provon 190, and BevWise A-100W. The two Barpro powders have

levels of calcium above the desired range. In fact, the Barpro 585 has 2.1grams of calcium per 100 grams powder. It was observed that Barpro 585 formed the least stable matrices with all fat types. While it is not desired by any particular theory of operation, it helps support the connection that high calcium amount might repel
5 fat:protein interactions by making the proteins more hydrophilic. Thus, calcium content can also be considered in preparing a protein fat matrix.

Avonlac 180, Provon 190, and BevWise A-100W, differed from the other proteins tested with regard to their contents of three amino acids: aspartic acid, threonine, and tyrosine. Both aspartic acid's R-group and threonine's R-groups are
10 considered polar. Tyrosine is the only amino acid of the three with a hydrophobic R-group. Thus, amino acid side chains and overall protein hydrophobicity can also be considered in preparing a protein fat matrix.

When looking at the lipid-protein matrix, the connections between the types of fat and the protein types determines how much residual fat leaves the matrix. Cocoa
15 butter and palm kernel oil appear to have low residual fat particularly when tested with the Provon, Avolac, and BevWise proteins. All of the Barpro 585 seems to have low fat retention for those that are higher in saturated fats and seems to be very high with canola oil. Both of the Barpros seems to work opposite to the other three types of proteins. Cocoa butter and palm kernel oil are cited as two fats that are not
20 recommended for tandem use in a food system because cocoa butter forms beta while PKO forms beta-prime crystalline structures. While coconut oil's most common binding crystallization forms are reported as beta-prime and alpha. (Ribeiro and others 2015) While it is not desired to be bound by any particular theory of operation, it might be the reason books and chocolate producers suggest that if other lipids are
25 mixed with chocolate, the added fast must be less than 5% of the total fats. However, within the formation of representative fat and protein matrices of the presently disclosed subject matter, a mixture of PKO and cocoa butter closer to 10 percent PKO as shown to be desirable.

Based on the evidence presented, it is shown that the presently disclosed
30 subject matter for the production of stable fat/protein matrices is practically useful and effective across a range of food systems. In some embodiments, the presently disclosed subject mixture comprises a stable protein in fat matrix comprising highly saturated lipids mixed with whey protein isolate. In one aspect, it was observed that

substitution of whey concentrate with ultra-filtration results in less desirable protein-fat interaction. In a particular representative embodiment, the blend of fat and protein ratio was 50/50, using a mixture of cocoa butter and palm kernel oil. Using these saturated fats together in this matrix does not cause the fat bloom that typically occur
5 with fat mixtures having dissimilar crystalline structures. In other particular embodiments, the moisture content of the WPI was observed to be below 5.0%. Other representative parameters for the composition include controlling pH between 6.5 and 3.5, calcium levels below 900 mg per 100 grams protein, relatively low sugar levels, high aspartic acid and threonine levels, and low tyrosine levels. Finally, a protein
10 particle size between 10 μ m and 130 μ m was observed to be desirable in some embodiments. In some embodiments, the construction of the matrix begins with melted fat incubated for 1-3 minutes before using a low shear mixing method in either a vertical or horizontal mixer followed by using a heated slab roller. Thus, aspects of the presently disclosed subject matter involve fat/protein ratios, such as 50/50
15 fat/protein ratios; particular combinations of specific fats and protein, such as whey proteins; particular amino acid compositions in the proteins used; particular protein particle size ranges; the general desirability of whey proteins over mixed casein and whey; the ability of cocoa and palm kernel fats to work together effectively in this system; and the mixing and molding methods used in the bar model.

20 IV. REFERENCES

All references listed below, as well as all references cited in the instant disclosure, including but not limited to all patents, patent applications and publications thereof, and scientific journal articles are incorporated herein by reference in their
25 entireties to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

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It will be understood that various details of the presently disclosed subject matter may be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

5

CLAIMS

What is claimed is:

1. A composition comprising a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group consisting of a
5 macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further optionally, wherein the dispersion is a uniform dispersion.
2. The composition of claim 1, wherein the macronutrient is a protein, a carbohydrate, a second type of fat in addition to the one forming the primary fat
10 matrix, water, or a combination thereof.
3. The composition of claim 1 or claim 2, wherein the fat matrix comprises a fat that is a solid at temperatures ranging up to 45°C.
4. The composition of claim 1 or claim 2, wherein the fat matrix comprises a fat that is semi-solid at temperatures ranging up to 40°C.
- 15 5. The composition of any one of claims 1-4, wherein the macronutrients are proteins and fats and the proteins and fats are present at ratio of between 1:1 and 1:3 protein to fat by weight.
6. The composition of any one of claims 1-5, wherein the macronutrient is a protein of about 18 to 25 kiloDaltons.
- 20 7. The composition of any one of claims 1-6, wherein the ingredient comprises particles and the ingredient particles have a particle size ranging from about 10 microns to about 200 microns.
8. The composition of any one of claims 1-7, wherein the ingredient has a D90 value for particle size of about 100 microns or less.
- 25 9. The composition of any one of claims 1-8, having a caloric value ranging from about 4 kilocalories per gram to about 8 kilocalories per gram.
10. The composition of any one of claims 2-9, wherein the protein comprises predominantly hydrophobic amino acid side chains, as determined by a

technique selected from the group consisting of direct sequencing of amino acids, Wimley-White interface or octanol scales, water micro-droplet contact angle measurements, other common measures of peptide and protein hydrophobicity, or any combination of the foregoing.

5 11. The composition of any one of claims 2-10, wherein the protein comprises a whey protein, a casein protein, an egg white protein, an insect protein, a plant protein, or combinations thereof.

12. The composition of any one of claims 2-11, wherein the protein
10 comprises at least about 20% of the caloric value of the composition, optionally at least about 25% of the caloric value of the composition.

13. The composition of any one of claims 2-12, wherein the carbohydrate comprises a sugar, a sugar syrup, a polyol, a flour, a starch, a fiber or hydrocolloid, or combinations thereof.

14. The composition of any one of claims 1-7, further comprising an
15 ingredient selected from the group consisting of a plasticizer, a thickener, an antioxidant, a chelator, a flavoring, and combinations thereof.

15. The composition of any one of claims 1-14, wherein the fat is a fat selected from the group consisting of cocoa butter, coconut oil, palm kernel oil, tristearin, and fractions and combinations thereof.

20 16. The composition of any one of claims 1-15, having an average water activity below 0.85, optionally below 0.75.

17. The composition of any one of claims 1-16, wherein the composition
is provided in the form of a bar, a filling, a ganache, a pastry, a shortbread, an icing, a yogurt, a butter, a gel, a paste, a pellet, a sphere (with or without a food-grade candy
25 shell), or an ovoid disc (with or without a food-grade chocolate, candy or gelatin shell/encapsulation).

18. A process for preparing a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group consisting of a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and

combinations thereof, further optionally, wherein the dispersion is a uniform dispersion, the process comprising:

- (a) heating the fat matrix to provide a melted or heat-plasticized fat matrix;
- (b) mixing the ingredient into the melted or heat-plasticized fat matrix,
5 optionally wherein the ingredient comprises particles; and
- (c) cooling the mixture formed in step (b) to form the composition comprising a dispersion of an ingredient in a fat matrix, optionally wherein the ingredient is selected from the group consisting of a macronutrient, a micronutrient, a dietary bioactive, a pharmaceutical ingredient, and combinations thereof, further
10 optionally, wherein the dispersion is a uniform dispersion.

19. The process of claim 18, wherein the fat matrix comprises a fat that is a solid at a temperature ranging up to 45°C.

20. The process of claim 18, wherein the fat matrix comprises a fat that is semi-solid at temperatures ranging up to 40°C.

- 15 21. The process of any one of claims 18-20, wherein the fat is a fat selected from the group consisting of cocoa butter, coconut oil, palm kernel oil, tristearin, and fractions and combinations thereof.

22. The process of any one of claims 18-21, wherein the macronutrient is a protein, a carbohydrate, a fat, water, or a combination thereof.

- 20 23. The process of any one of claims 18-22, wherein the macronutrients are proteins and fats and the proteins and fats are present at ratio of between 1:1 and 1:3 protein to fat by weight.

24. The process of any one of claims 18-23, wherein the macronutrient is a protein of about 18 to 25 kiloDaltons.

- 25 25. The process of any one of claims 18-24, wherein the ingredient has a particle size ranging from about 10 microns to about 200 microns.

26. The process of any one of claims 18-25, wherein the ingredient has a D90 value for particle size of about 100 microns or less.

27. The process of any one of claims 18-26, having a caloric value ranging from about 4 kilocalories per gram to about 8 kilocalories per gram.
28. The process of any one of claims 22-27, wherein the protein comprises predominantly hydrophobic amino acid side chains, as determined by a technique
5 selected from the group consisting of direct sequencing of amino acids, Wimley-White interface or octanol scales, water micro-droplet contact angle measurements, other common measures of peptide and protein hydrophobicity, or any combination of the foregoing.
29. The process of any one of claims 22-28, wherein the protein comprises
10 a whey protein, a casein protein, an egg white protein, an insect protein, a plant protein, or combinations thereof.
30. The process of any one of claims 22-29, wherein the protein comprises at least about 20% of the caloric value of the composition, optionally at least about 25% of the caloric value of the composition.
31. The process of any one of claims 22-30, wherein the carbohydrate
15 comprises a sugar, a sugar syrup, a polyol, a flour, a starch, a fiber or hydrocolloid, or combinations thereof.
32. The process of any one of claims 18-31, further comprising an ingredient selected from the group consisting of a plasticizer, a thickener, an
20 antioxidant, a chelator, a flavoring, and combinations thereof.
33. The process of any one of claims 18-32, having an average water activity below 0.85, optionally below 0.75.
34. The process of any one of claims 18-33, comprising holding the melted or heat-plasticized fat matrix for a period of time prior to the mixing.
35. The process of claim 34, wherein the holding of the fat matrix is carried
25 out for 1 to 3 minutes.
36. The process of any one of claims 18-35, mixing the ingredient into the melted or heat-plasticized fat matrix using a low shear mixing method.

37. The process of any one of claims 18-34, comprising forming the composition into a bar, a filling, a ganache, a pastry, a shortbread, an icing, a yogurt, a butter, a paste, a gel, a pellet, a sphere (with or without a food-grade chocolate, candy or gelatin shell/encapsulation), or an ovoid disc (with or without a food-grade
5 chocolate, candy or gelatin shell/encapsulation).

38. The process of claim 37, wherein forming the composition comprises forming the composition on a slab roller.

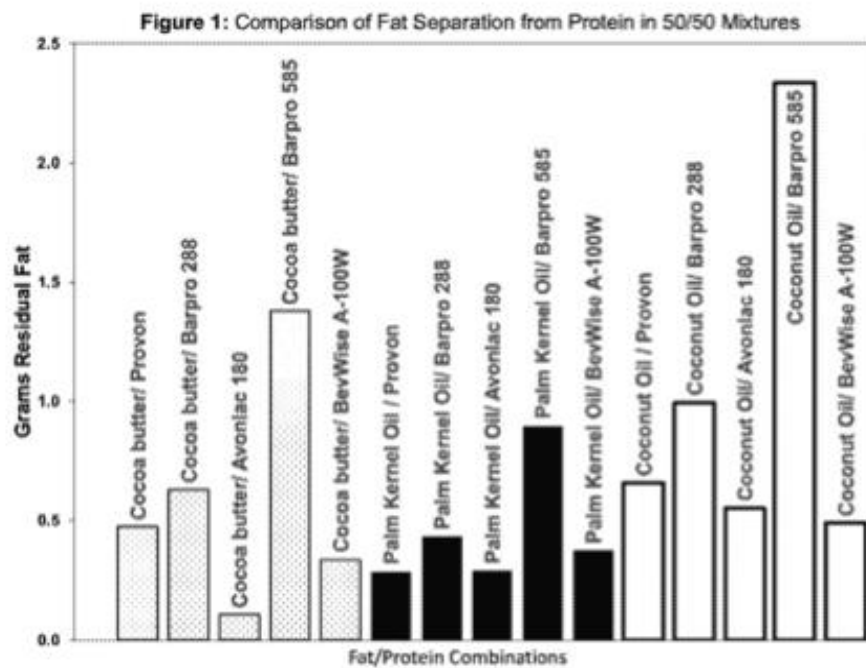


Fig. 1