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Chemical Properties And Probiotics Survivability Of Symbiotic Coconut Yogurt Using Polymerized Whey Protein As A Gelation Agent

Jinglin Lu
University of Vermont

CHEMICAL PROPERTIES AND PROBIOTICS SURVIVABILITY OF SYMBIOTIC COCONUT YOGURT USING POLYMERIZED WHEY PROTEIN AS A GELATION AGENT

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Jinglin Lu

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Mingruo Guo, PhD., Advisor
Jana Kraft, Ph.D., Chairperson
Todd Pritchard, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

Abstract

Food products from coconut fruits are delicious and nutritious; the coconut water could be directly used as a sports drink; whereas the meat can be processed to coconut milk, coconut puree and other products that are rich in medium fatty acids that can be burnt quickly to provide energy to the body. In recent years, products that incorporated the probiotics with coconut have been developed, however the texture and nutritional qualities were not satisfied.

The purpose of the study was to create a functional coconut yogurt using polymerized whey protein (PWP) as a gelation agent to achieve a better mouthfeel and also increase the protein content of the final product. In the preliminary studies, different ratios of PWP/stabilizer system were tested to optimize the formulation. After the formula was finalized, three groups including control yogurt, PWP fortified yogurt and Ca/vitamin D fortified yogurt of 3 trials were made for further analysis.

The chemical composition of the coconut yogurt including protein, fat, minerals, total solids and ash were analyzed. A sensory evaluation was also conducted to compare the lab samples from each group with the commercial samples. Result showed that there was no difference of yogurt properties within three lab groups except for the firmness. However, the lab groups had a significantly ($P<0.05$) higher score for most properties than commercial group.

pH values, viscosity and probiotics survivability were also analyzed weekly during the 8 weeks of storage. The pH significantly increased ($P<0.05$) for all groups, whereas the viscosity showed a slow but significant decrease in all groups ($P<0.05$) with the same decreasing rate ($P>0.05$). At the last week of study, only *Lactobacillus paracasei* were above the therapeutic level in all three groups. *Lactobacillus acidophilus* did not reach that level at the first week of storage. The difference of survivability among three groups were all significant different ($P<0.05$).

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Abbreviations

EPSs: exopolysaccharides

DE: degree of methyl esterification

GABA: γ-aminobutyric acid

HGA: homogalacturonan

HM: high methoxyl

LA: *Lactobacillus acidophilus*

LB: *Lactobacillus delbrueckii subsp. bulgaricus*

LM: low methoxyl

LP: *Lactobacillus paracasei*

MCFAs: medium chain fatty acids

PWP: polymerized whey protein

RG-I: rhamnogalacturonan I

RG-II: rhamnogalacturonan II

ST: *Streptococcus thermophilus*

Literature Review

Chapter 1 Starter Culture and Probiotics

1.1 Starter Cultures

Starter culture is the core of all variety of fermenting products. In this study, the YoFlex® YF-L02 DA from Chr Hansen has been applied to make the coconut milk yogurt. It contains the mixed starter culture *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii subsp. bulgaricus* (LB) as well as probiotics including *Bifidobacterium* species, *Lactobacillus acidophilus* (LA), *Lactobacillus paracasei* (LP). Most of the starter culture only consist of lactobacillus, however, some exceptions may involve *Lactococcus* and/or yeasts. The starter culture can be a single strain starter, such as ST alone. It can also be a mixed culture, such as ST and LB. Sometimes the probiotics will be mixed with the starter culture to achieve certain health benefits. However, to be named yogurt, the product must include both ST and LB (Chandan & Kilara, 2013). The commensal relationship between them will significantly accelerate the process of fermentation (Moon & Reinbold, 1976).

1.1.1 Functions of Starter Cultures

The primary function of starter culture is to convert the lactose or other carbohydrates to lactic acid by fermenting the substrate. As the lactic acid accumulates, the physiochemical of the substrate starts to change, and when the lactic acid reaches a lower

level, it presents a preservative effect that can inhibit many unwanted microbes (Chandan & Kilara, 2013).

The second function of starter culture is giving the special flavor and texture to the yogurt by producing metabolites, including diacetyl, acetaldehyde, ethyl alcohol, carbon dioxide and exopolysaccharides (EPSs) (Yildiz, 2010).

1.1.2 The interaction between starter culture

The LB and ST are commensal to each other, and this combination can accelerate the yogurt fermentation and pH dropping. At the first stage of fermentation with the inoculation of starter culture, ST grows faster and release metabolites, including lactic acid, carbon dioxide, and formic acid (Chandan & Kilara, 2013). As a synergistic bacteria, LB is stimulated to grow later as the pH drops to around 5 (Beshkova, 1998)(Lynradke & Sandine, 1983). Then, the LB produces a large amount of stimulatory peptides and amino acids through proteolysis to stimulate the growth of ST (Moon & Reinbold, 1976). Then the ST is inhibited as the lactic acid reaches a certain concentration. The LB is responsible for further decreasing the pH to 4 (Yildiz, 2010).

1.2 Probiotics

Unlike ST and LB, probiotics can tolerate the gastric acid, bile salts and digestive enzymes and finally reach the intestine, thereby carry out the health benefits to human body by colonizing living cells and producing the metabolites. On the current market, *L. acidophilus, L. casei, L. paracasei, L. rhamnosus, L. plantarum, Bifidobacterium* are the most common probiotics that have been added to the yogurt. The health effects of those

probiotics are well documented. However, to ensure there are enough bacteria to reach and colonize in the intestine and achieve certain health benefits, Linares *et al.* (2017) suggest taking yogurt with probiotics at a therapeutic level of 10^6 CFU/g. It is suggested that the probiotics should be taken regularly to maintain the health benefit; since the colonization of probiotics only lasts a few weeks, the microflora will restore the original condition (Fukushimaa, Kawatab, Harac, Teradac, & Mitsuoka, 1998).

1.2.1 Bifidobacterium

Bifidobacterium is identified as a Gram-positive, non-spore-forming genus that has fructose-6-phosphate phosphoketolase (De Vos et al., 2009) to metabolize carbohydrates. Most of the strains can digest different types of sugar as their carbon source, from polysaccharides to monosaccharides. Theoretically, the genus produces acetic and lactic acids at a molar ratio of 3:2(Chandan & Kilara, 2013). Bifidobacterium is obligate anaerobic and nutritiously fastidious with an optimal growth pH at 6.0-7.0, whereas the pH of yogurt is below 5.5, which is out of the pH range for the growth of the bifidobacteria (De Vos et al., 2009). It is difficult for those bacteria to grow during the yogurt fermentation. Bifidobacterium is not only the species that firstly colonize in our intestine from being born, but also the normal inhabitants in the human GI tract that play a crucial role on human health (Mitsuoka, 1984). The ratio of each species within the Bifidobacterium genus and the ratio of the Bifidobacterium genus to the total intestinal microflora are changing throughout the lifetime (Mitsuoka, 1984). The Bb-12 strain used in this study is one of the most documented strains in all potential probiotics. In a small

sample study, 7 healthy infants from 15-31 months old were fed infant formula containing *Bifidobacterium bifidum* strain Bb12 for 21 days and several health-promoting results were observed; the gut microflora composition was positively shifted, and the normal microflora metabolic activities were also improved. Also, since the short-chain fatty acids level was elevated, the pH of large intestine had decreased consequently. As a result, the putrefactive products would be neutralized or inhibited (Fukushima, Li, Hara, Terada, & Mitsuok, 1997).

1.2.2 *Lactobacillus acidophilus*

L.acidophilus is a normal flora commonly found in human mouth, vagina and GI tract. They are gram-positive, obligate homofermentative and produce both L(+) and D(-) lactic acid (De Vos et al., 2009). It is a well-documented probiotic species with numerous health benefits. LA are commonly used with starter culture to make probiotic yogurt. The end products from LA are lactic acid, acetic acid and H_2O_2, which make the intestinal environment become less favorable for pathogens. The strain LA-5 that used in our study also produces bacteriocin CH5 and this compound shows the antagonistic effect toward many pathogenic bacteria as well as yeast/mold in a wide range, including Gram-positive *L. monocytogenes,* Gram-negative *E. coli* as well as the fungi *Candida sp*. (Chumchalova, Stiles, Josephsen, & Plockova, 2004).

1.2.3 *Lactobacillus paracasei*

L. paracasei are gram-positive, facultatively heterofermentative lactic acid bacteria that can tolerate the oxygen and produce L lactic acid with few strains produce the

inactive form (De Vos et al., 2009). They also have a wide growth temperature that ranges from 10-40℃ and even at 5℃ for some strains. LP can be found in human intestinal tract and mouths as a natural microflora. However, due to the unhealthy modern diet mode, which tends to intake more refined food and meat but less vegetable and fruits, these health promoting bacteria are reducing or disappearing in the human gut (Hentges, 1983). Taking LP from either a capsule or yogurt could significantly decrease the number of *C. dificile (Sullivan, Bennet, Viitanen, Palmgren, & Nord, 2009)*. Many studies showed that LP is a robust bacteria. During the yogurt storage, the number of LP was showing slightly increased or no significant change (Lee & Seppo, 2009).

1.3 Probiotics and Human Health

In a modern diet, the increased intake of processed food and lacking fresh fruits and vegetable intake may disturb the balance of the natural microflora, especially the Lactic Acid Bacteria (LAB) (Cho & Finocchiano, 2016). If the number of pathogenic bacteria increases in the intestine, more toxic chemicals and harmful enzymes can be released and eventually absorbed into the body. For the sake of health maintenance, it is suggested to take probiotics or fermented food regularly to replenish the normal microflora (Malik et al., 2018).

It is generally accepted that both LB and ST are not probiotics since they cannot survive from the harsh condition of the GI and get to the large intestine. However, people could still benefit from the metabolites and bioactive compounds that ware produced from them during the fermentation process. For example, the exopolysaccharides (EPSs)

that are produced by the ST are showing a prebiotics effect on human health (Chandan & Kilara, 2013). It also presents an anti-inflammatory, anti-proliferative effect on human health (Li & Shah, 2016). Whereas probiotics can tolerate the harsh gut environment, then arrive and colonize in the intestine in a viable form. Chandan (2013) summarized the major mechanisms that are associated with the health benefits of probiotics as: "1. Competition with other microflora for nutrients; 2. Production of organic acids to inhibit enteropathogens; 3. Production of bacteriocins and metabolites that either benefit health or inhibit pathogens; 4. Immunomodulation; 5. Adhesion to intestinal mucosa competing with pathogens" (Chandan & Kilara, 2013)

Nutritional Aspect

The probiotics can promote nutrition intake by several mechanisms, such as producing the vitamins, increasing the bioavailability. In a study, 24 strains from 5 strains of *Bifidobacterium* genus were analyzed for their ability to produce the water-soluble vitamins. It was concluded that most strains could synthesize thiamine, nicotinic acid, folic acid, pyridoxine and vitamin B_{12} at different rates and levels, depending on the species (Deguchi, Morishita, & Mutai, 1985). The *Bifidobacterium* was also reported to enable to produce the biotin (Noda, Akasaka, & Ohsugi, 1994).

Immunomodulatory and Anti-tumor Effects

Some probiotic species could improve the nonspecific immune responses. A human studied that was focused on the immunomodulating effect of probiotics demonstrated that consumption of yogurt that contained either LA or *B. bifidum Bb 12* could significantly enhance the phagocytosis of leukocytes (SCHIFFRIN, ROCHAT, LINK-AMSTER,

AESCHLIMANNP, & DONNET-HUGHES, 1995). Comprehensive research that was investigating the relationship between probiotics and breast cancer summarized the anti-cancer effect of probiotics (Malik et al., 2018). It was observed that healthy breast tissue has more *Lactobacillus* and *Streptococcus* colonized. While those two genera possess anticarcinogenic effects, which include stimulating the production of NK cells to regulate the tumor progression, producing antioxidants to reduce the DNA damage and breaking down the carcinogens. The study suggests that it is beneficial for both healthy people and especially cancer prognostic patients to take supplements or food with probiotics to promote the normal immune system.

Anti-bacteria and Anti-virus Effect

Both *L. acidophilus* and *Bifidobacterium* have antagonistic effects on those common pathogenetic bacteria, including *S. aureus*, enteropathogenic *E. coli* and *C. perfringens*; In children, it is confirmed by studies that probiotics combining *S. thermophilus* and *B. animalis* Bb-12 can prevent diarrhea that caused by rotaviruses in young children that aged from 5-24 months, it also helps to shorten the period of the symptom (Saavedra, Bauman, Oung, Perman, & Yolken, 1994). In adult studies, the *Bifidobacterium* showed a preventive effect and symptoms improvement for traveler's diarrhea as well as the antibiotics-induced diarrhea (Lee & Seppo, 2009).

Probiotics and Antimutagenicity

Cassand *et al*. (2002) conducted an experiment to investigate the antimutagenic activity of *Bifidobacterium* and *L. acidophilus* on 8 types of mutagens that commonly found in our food. The study suggests that the live probiotic cells have a higher

antimutagenicity than the killed group, which implies that the living cells may permanently metabolize the toxins to non-toxic compounds; The metabolites, such as organic acids that produced by probiotics also have binding and inhibiting effects on mutagens. Amongst, the butyric acid has the highest inhibiting effect and followed with acetic acid (Tavan, Cayuela, Antoine, & Cassand, 2002). This implies that consuming live probiotics regularly may promote wellbeing by neutralizing mutagens.

1.4 Factors Affecting the Viability of Probiotics

There are many factors that may affect the survivability. Firstly, the packaging is one of the most important factors. Most LAB prefer an environment with a low oxygen saturation. Study shows that the cup with gas-barrier could effectively prevent the oxygen from entering the yogurt and as a result, the probiotics survivability was significantly higher than the group without the gas-barrier (Miller, Nguyen, Rooney, & Kailasapathy, 2002). Secondly, the yogurt ingredient may positively or negatively influence the survivability. Shah *et al.* reported that addition of 250mg/L cysteine in the milk substrate could significantly increase the counting number of LA compare to control yogurt, whereas the *Bifidobacterium* count showed inhibited (Dave & Shah, 1997b). Thirdly, the storage temperature is a very important factor that affect the probiotics viability. Ferdousi *et al.* tested different probiotics that exposed to 20℃ for 24 hours, including L. *rhamnosus*, L. *paracasei*, L. *acidophilus* and B. *lactis*, and result showed that all testing species had a significantly decreased survivability, and the B. *lactis* is more susceptible to the temperature than other species (Ferdousia et al., 2013). This study demonstrated that

to keep a good probiotics survivability, it is important to avoid the longtime exposure to the high temperature (>20℃) during the transportation and/or storage. Moreover, other factors such as the interaction between the starter culture and probiotics, chemical composition of the fermenting substrate should also be taken into consideration (Lee & Seppo, 2009).

Chapter 2 Chemical Composition of Coconut Fruit

Coconut tree is one of the most valuable commodities in tropical area. Coconuts oil, coconut water, coconut milk, copra and coconut fibers are the main products from coconut that can be found everywhere in our daily life. The coconut meat and coconut water are highly nutritious and tasty.

2.1 Coconut Meat

Coconut meat is extracted from the endosperm tissue of the coconut fruits. The nutrients composition of coconut meat is changing greatly at different maturation stage (Santoso, Kubo, Ota, Tadokoro, & Maekawa, 1995). The most featured nutrient composition is the medium chain fatty acids (MCFAs), which account for about 50% of the total lipid content in the mature coconut meat.

The **Table 1** shows that the coconut meat does not contain much proteins, however, the coconut proteins have high total essential amino acid (Yan Li, Yajun Zheng, Yufeng Zhang, Jianguo Xu, & Gang Gao, 2018) . The arginine and glutamic acid are the most abundant amino acids in the coconut meat. The coconut proteins consist of albumin,

prolamin, globulin, glutelin-1 and glutelin-2; The glutelin-1, glutelin-2 and prolamin showed strong antioxidant effect (Y Li, Y Zheng, Y Zhang, J Xu, & G Gao, 2018).

Study shows the coconut kernel protein could decrease the blood sugar level, elevate the insulin secretion and increase the glycogen for the alloxan-induced diabetic rats, and it is the arginine played an major role on those improvement (Salil, Nevin, & Rajamohan, 2012). The arginine has been reported to be a precursor for the synthesis of many compounds including NO, glutamate, urea, creatine, proline and agmatine (Visek, 1986). Moreover, the arginine can stimulate the secretion of insulin, growth hormone and glucagon. (Visek, 1986; Wu & Morris, 1998). The arginine could also help to regenerate the new pancreatic tissue and repair the damaged one (Wu & Morris, 1998).

2.2 Coconut Water

Coconut water has long been used as a plant tissue cultivation and growth supplement (Tulecke, 1961). **Table 1** shows the coconut water composition. Both young coconut water and mature coconut water are rich in minerals, carbohydrates, and trace amount of fatty acids and protein, which makes the coconut water to become a good resource to replenish the minerals and carbohydrates that lost during the exercise (Yong, Ge, Ng, & Tan, 2009). Coconut water is particularly high in potassium but low in sodium. Study suggested that keep a diet with a lower sodium and higher potassium may help to ameliorate the hypertension symptom (Suter, 2002). The fresh coconut water has a small amount of vitamin C and B vitamins (Santoso et al., 1995). Those vitamins are very susceptible to processing procedures, especially heating treatment.

Moreover, Mandal *et al*. (2009) identified 3 antimicrobial peptides, which are named Cn-AMP1, Cn-AMP2 and Cn-AMP3, from the young coconut water, with molecular mass under 3 kDa and showing a strong inhibition of both Gram-positive and Gram-negative bacteria as well as fungi. These peptides, especially Cn-AMP1, could play a role on the non-specific innate immune system to defense the host from getting microbial invasion (Mandal et al., 2009). Further study reported that the Cn-AMP1 also share a character of inhibition on the human epithelial colorectal adenocarcinoma cells (Caco-2) with AMPs from other plants (Osmar et al., 2012).

The antioxidant compounds were also detected in the coconut water (not shown in the table). Tan *et al.* analyzed that the level of total phenolics content in coconut water ranges from 25 to 54mg/L, depending on the maturity stage (Tan, Cheng, Bhat, Rusul, & Easa, 2014). The phenolic compounds helps to neutralize the ROS and therefore protect the cells from excessive oxidative damage (Collins, 1999). The coconut water also contains organic acid, especially malic acid and citric acid and trace amount of pyridoline as well as shikimic and quinic acids were also detected (Yong et al., 2009). Phytohormones are detected from the young coconut, including cytokinins and kinetin and these are crucial factors for promoting the plant tissue development and growth (Ge et al., 2007; Santoso et al., 1995; Tulecke, 1961). In human nutrition and health aspects, the kinetin was reported to have an anti-aging effects on human skin cells (Rattan & Clark, 1994). Also, Olsen *et al.* indicate that the kinetin could protects the DNA from oxidative damage both *in vivo* and *in vitro* due to its anti-oxidative property (Olsen, Siboska, Clark, & Rattan, 1999).

Table 1. Chemical Composition of Coconut Meat and Coconut Water

	YM	MM	YW	MW
General chemical composition (Santoso et al., 1995)				
Dry matter (DM) %	7.58	52.29	5.82	5.55
Ash (%DM)	7.94	1.15	14.89	8.42
Crude protein (%DM)	16.0	7.10	2.10	9.36
Total lipid (%DM)	20.22	62.64	1.26	2.67
Carbohydrate (%DM)	54.9	29.1	81.8	79.5
Lignin (%DM)	0.97	6.69	/	/
Hemicellulose (%DM)	3.09	6.73	/	/
Cellulose (%DM)	8.09	7.09	/	/
Pectin (%DM)	2.65	0.74	/	/
Sugar and organic acid composition (Santoso et al., 1995)				
Sucrose (%DM)	15.3	4.77	1.02	9.24
Glucose (%DM)	2.85	0.24	44.9	26.7
Fructose (%DM)	3.22	0.46	43.9	25.7
Acetic acid (mg per 100g DM)	/	/	/	1.3
Tartaric acid (mg per 100g DM)	/	/	1.6	2.4
Citric acid (mg per 100g DM)	63.0	62.3	/	24.8
Malic acid (mg per 100g DM)	2210	740	317	307

YM=young meat, MM=mature meat, YW=young water, MW=mature water

Continued：	**YM**	**MM**	**YW**	**MW**
Vitamins (mg per 100g dry matter) (Santoso et al., 1995)				
Vitamin B_1	0.26	0.10	Trace	0.01
Vitamin B_2	0.14	0.02	0.01	0.01
Vitamin B_6	0.41	0.14	/	/
Niacin	11.6	1.49	/	/
Vitamin C	37.8	5.27	7.41	7.08
α-Tocopherol	38.1	0.94	/	/
γ-Tocopherol	0.29	0.05	/	/
Mineral composition (Santoso et al., 1995)				
Calcium (%DM)	0.29	0.03	0.47	0.57
Magnesium (%DM)	0.43	0.12	0.11	0.17
Potassium (%DM)	4.47	0.68	3.50	4.64
Sodium (%DM)	0.10	0.02	0.03	0.29
Phosphorus (%DM)	0.39	0.19	0.08	0.23
Sulphur (%DM)	0.15	0.11	0.01	0.07
Manganese (ppm)	42.9	16.4	20.3	14.4
Iron (ppm)	43.6	35.9	4.06	2.94
Zinc (ppm)	36.5	17.8	11.3	3.51
Copper (ppm)	6.19	36.2	0.96	5.32
Boron (ppm)	7.73	3.34	8.49	14.3
Aluminum (ppm)	9.60	5.06	12.8	11.6

YM=young meat, MM=mature meat, YW=young water, MW=mature water

Continued:	**YM**	**MM**	**YW**	**MW**
Fatty acid composition (% of total lipids) (Santoso et al., 1995)				
8:0	0.03	4.34	/	/
10:0	0.07	6.22	0.95	1.90
12:0	2.25	48.6	2.7	18.5
14:0	3.99	19.2	3.16	12.8
16:0	22.5	9.64	29.8	21.6
16:1	/	/	1.54	0.98
17:0	/	/	1.18	1.06
18:0	0.04	3.23	5.28	7.28
18:1	38.3	7.18	26.5	20.4
18:2 n=6	32.6	1.59	15.5	4.36
20:0	/	/	2.19	2.23
20:1	/	/	6.63	2.63
20:4 n=6	/	/	1.89	2.96
22:1	/	/	1.47	3.10
Amino acid composition of coconut proteins (mg/g of defatted sample)				
Asp	8.96	12.6	1.60	7.89
Thr	3.43	4.69	0.20	2.22
Ser	5.72	7.09	0.64	4.00
Glu	19.9	27.5	3.44	13.6
Pro	3.93	4.98	0.52	3.01
Gly	4.61	5.72	0.43	1.39
Ala	9.18	7.10	1.13	6.33
Val	4.75	6.76	0.91	3.98
Met	1.65	2.53	0.22	1.48

Continued:	**YM**	**MM**	**YW**	**MW**
Ile	3.30	4.59	0.26	1.53
Leu	6.74	9.17	0.66	2.05
Tyr	1.74	2.70	0.00	1.42
Phe	4.01	5.73	0.26	1.14
His	3.08	4.10	0.39	2.44
Lys	5.28	17.4	4.72	1.78
Arg	16.8	24.3	0.13	6.80

YM=young meat, MM=mature meat, YW=young water, MW=mature water

2.3 Medium Chains Fatty Acids

The Medium Chain Fatty Acids (MCFAs) are the most predominant nutritious character found in coconut meat. It is generally believed that the MCFAs contain 6 to 12 carbons (Dayrit, 2014) and they have unique metabolic pathways that are different from those long chain fatty acids. From the **Table 1**, the MCFAs make up approximately 60% of the total coconut fatty acids, of which the lauric acid (12:0) accounts for about 50%.

When the MCFAs were consumed, the lingual and gastric lipase act efficiently on the triglycerides with medium chain fatty acids. As a result, the digestion of MCFAs in the stomach is easier than the long chain triglycerides (Sareen, Gropper, & Smith, 2016). In the small intestine, the medium chain triglycerides or diglycerides can be preferably and completely hydrolyzed by the pancreatic lipase (Bach AC, 1982).

Not like saturated LCFAs, which need to be incorporated into chylomicrons and enter the lymphatic system, most of the MCFAs could pass the mucosal cell and enter the

bloodstream of the portal vein and sent to liver as free acids (Bloom, Chaikoff, & Reinhardt, 1951). Therefore, the MCFAs can be burnt as a highly efficient fuel that provides energy to the body.

In the liver, the MCFAs can rapidly enter the mitochondrial bi-layer membrane by passive diffusion without the assistance from carnitine like LCFAs do (Dayrit, 2014). Study indicates that the diffusion rate can be 100 times faster for a carbon chain with two carbons shorter (Hamilton, 1998). In the mitochondria, β-oxidation is the major pathway to metabolize the fatty acids. There are four acyl-CoA dehydrogenase enzymes that are responsible for the oxidation of fatty acid with different chain length (Sareen et al., 2016). MCT such as lauric acid can be broken down very fast in the mitochondria since two of the enzymes act highly efficient on it (Wanders, 1999). Then the acetyl-CoA can be converted to ketone bodies, including acetoacetic acid and its derivates beta-hydroxybutyric acid and acetone (Dayrit, 2014). Thus, coconut oil is a good choice for people on a keto diet.

Fernando *et al*. summarized that since the MCFAs are not likely to form lipoproteins, also they do not prefer the esterification, so the MCFAs are not likely to contribute to the body fat deposit as well as the building of lipoprotein in the artery walls (Fernando et al., 2015). However, an early study showed that there is still one third of MCFAs will enter the lymph and incorporated into chylomicrons (Bragdon & Karmen, 1960); Moreover, Larry *et al*. also found that the ratio of incorporation of MCTs into chylomicron has increased after feeding the MCT diet in a consecutive 6 days (Swift, Hill, Peters, & Greene, 1990).

Moreover, the antimicrobial properties of lauric acid and monolaurin has been extensively studied both *in vitro* and *in vivo*. Dayrit (Dayrit, 2014) summarized that among all the saturated fatty acids, the lauric acid present the most inhibition to the gram positive bacteria and certain viruses and fungi. A dental study shows that the lauric acid has antiplaque potential (Schuster, Dirksen, & Ciarlone, 1980). It is also reported that the monolaurin, a derivative of lauric acid triglyceride, has a stronger inhibiting effect than lauric acid (Kabara, 1979), and the monolaurin shows a strong activity on H. *pylori (Sun, Connor, & Roberton, 2003).*

Chapter 3 Nutrition Supplements

Human nutrients are generally divided into six categories, including protein, fat, water, vitamins, dietary fibers and minerals. In modern diet mode, people tend to take too much refined food but less natural and fresh food, which may result in vitamins and mineral deficiency and the symptoms are not usually noticeable for a short period (Sareen et al., 2016). However, in the long run, the functions of those nutrients for human health are profound. Taking a regularly daily nutrients supplements are especially important for those who are not on a healthy diet, also people on specific diet, such as vegetarian or vegan.

3.1 Dietary Fibers

3.1.1 Definition and Classification

Dietary fibers refers to carbohydrates that cannot be digested by human digestive enzymes and they are rich in fresh fruits, vegetables, grains and beans (Sareen et al., 2016). Dietary fibers can be divided into two groups: soluble fibers and insoluble fibers. The soluble fibers are fructans, pectin, β-glucans, gums and pectin (depends on type); the insoluble fibers are lignin, cellulose, hemicellulose, pectin (depends on types). The soluble fibers are all fermentable, whereas the lignin and cellulose from the insoluble group are nonfermentable (Sareen et al., 2016). Below in **Diagram 1** is the a more visualized classification.

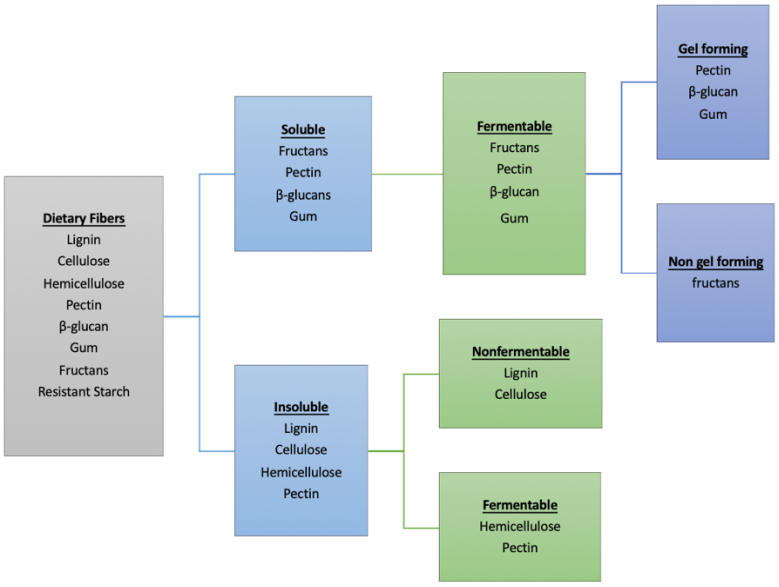


Diagram 1. Classification of Dietary Fibers

Different type of fiber brings about different health benefits. The gel-forming fibers could slow down the gastric emptying rate and particularly reduce the nutrient absorption of fat and carbohydrates and as a result, this type of fiber may lower the serum

triglycerides and cholesterol, also improve the glycemic response (McRorie & McKeown, 2017).

The fermentable fibers could soften the stool by increasing the fecal bacteria mass; Whereas the non-fermentable fibers, such as wheat bran, have a laxative effect since the fibers can absorb a large amount of water, thereby increase the volume of the stool and stimulate the defecation (Sareen et al., 2016).

In food industry, some fibers can be further processed and added to the food product acting as a gelation agent, thickener or stabilizer, such as pectin, guar gum. See Chapter 5 for more details.

3.2 Prebiotics

3.2.1 Definition

The dietary prebiotics was recently defined as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Davani-Davari et al., 2019).

From the definition, a prebiotic have to meet the following criteria: ① it should resist the low pH and human gastric enzymes and should not be absorbed； ② it can be fermented in the intestine; ③ it can selectively stimulate the growth and/or activity of the probiotics thereby promote the host's health (Gibson, 2010). The prebiotics have some advantages over probiotics. Firstly, they have much longer shelf life and do not require the cold storage. Secondly, they are very stable during the food processing procedures, such as heat treatment and acidification. Thirdly, they will not cause any bacteremia like probiotics do since they only stimulate the organisms that have already existed in the

intestine (Cho & Finocchiano, 2016; Lee & Seppo, 2009). We should notice that the dietary fibers and prebiotics are not the same. Firstly, the degree of polymerization (DP) of dietary fibers is at least 3; whereas the prebiotics can be found as disaccharide, for example: lactulose, which is formed from one fructose and one galactose; secondly, the dietary fibers cannot be hydrolyzed by the enzymes from the small intestine.

3.2.2 Classification and Mechanisms of Prebiotics

There are 5 types of prebiotics: ① Fructans, including inulin and fructo-oligosaccharide (FOS) and oligofructose ② Galacto-oligosaccharides (GOS) ③ Resistant starch and glucose-derived oligosaccharides ④ Other oligosaccharides, which can be derived from pectin ⑤ Non-carbohydrate oligosaccharides, include flavanols (Davani-Davari et al., 2019). The FOS can be found in artichoke, chicory and onions; the GOS is present in soybean; the green bananas are rich in resistant starch.

It seems that the bifidobacteria could always respond to almost all kinds of prebiotics，despite the chemical composition, molecular structure and size of the prebiotics (Lee & Seppo, 2009). Most of the health benefits of prebiotics are achieved by the increase of the bifidobacteria number, as well as their metabolizing activities and metabolites. In human adult study, it is observed that the *Bifidobacterium* population was increased by 10-100 folds during the period that were feeding prebiotics; also, the lactobacilli are proliferated by particular types of prebiotics, include lactulose and galactooligosaccharides. As a result of the increasing number of those good bacteria,

more vitamins and short-chain fatty acids are produced, and less pathogens are inhibited due to the competitive environment and the increased amount of antagonistic metabolites that are produced by the probiotics (Lee & Seppo, 2009).

3.2.3 Health Benefit of Prebiotics

Lactulose is one of the prebiotics that belongs to disaccharides that consist of one galactose and one fructose. It has been used as laxative to treat constipation and hepatic encephalopathy.

The GOS and/or FOS are commonly added to the infant formula in recent years. Compare to the traditional infant formula without the prebiotics added, the GOS/FOS fortified formula is showing significantly positive impacts on many aspects, including a more breast-fed like composition of *Bifidobacterium* and *Lactobacillus (Haarman & Knol, 2005)*; reduced number of pathogenic bacteria; more acidic and softer stool (Boehm et al., 2005) (Knol et al., 2005); higher frequency of defecation (Boehm et al., 2005); higher level of IgA in the intestine (Boehm et al., 2005).

Several human studies indicated that the inflammatory bowel disease (IBD) including Crohn's disease, ulcerative colitis and pouchitis could be ameliorated by intaking certain prebiotics. Several improvements were observed including a reduced mucosal inflammation, lowered mucosal pH, reduced pathogenic bacteria, regeneration of epithelial tissue, decreased mucosal proinflammatory cytokines and increased number of bifidobacteria in the stool (Lee & Seppo, 2009).

The inulin and fructo-oligosaccharide have demenstrated triglyceride-lowering and cholesterol-lowering effects in many animal studies and the proposed mechanism

involved the inhibition of *de novo* fatty acid synthesis and consequently the VLDL level will decrease (Williams & Jackson, 2007). Whereas the human studies showed mixed results. One proposed explanation is, in healthy human, the *de novo* fatty acid synthesis is usually supressed (Mashima, Seimiya, & Tsuruo, 2009); instead, the exogenous lipid is used to sythesize the hepatic triacylglycerol (Aarsland, Chinkes, & Wolfe, 1996).

It was demonstraed by many studies that the pectin may reduce the cholesterol level with a minimal daily dosage of 6 grams to come into play (Ginter, Kubec, Vozar, & Bobek, 1979; P Sriamornsak, 2001).

Though there are evidences from *the vitro* and animal studies showed that the prebiotics may reduce the risk of colon cancer, it lacks the evidence that collected from longterm human study (Cho & Finocchiano, 2016).

3.2.4 Dose and Side Effect

Taking prebiotics may bring some side effects. Bloating, cramping and osmotic diarrhea are the most common symptoms that caused by the change of osmosis from the microbial fermentation; And the short chain prebiotics appear to cause more side effects since they are fermented faster and mainly in the proximal colon; whereas the prebiotics with longer chain length are fermented much slower and in the distal colon (U. Svensson & Håkansson, 2013). The recommended effective dose ranges from 2.5-10 g per day (Davani-Davari et al., 2019).

3.2.5 Inulin

The inulin is one of the most common prebiotics and/or soluble dietary fiber on the market. It belongs to linear fructans that built from β-2, 1-D-fructose units linked by β

（2→1）glycosidic bonds and terminated by a D-glucose residue with α（2→1）linkage (Chi et al., 2011). It can be extracted from chicory root and further processed or modified into products with different degree of polymerization (DP) from 2 to 60. On average, the standard chicory inulin has a shorter chain length with 10-12 units; whereas the long-chain inulin contains no less than 25 units.

Inulin has been widely applied in all variety of food products as a dietary fiber supplement as well as a food additive. When added to yogurt or other fermented milk products, the inulin could improve the growth and survivability of the *L. acidophilus* and *Bifidobacterium* (Aryana, Plauche, Rao, McGrew, & Shah, 2007; Oliveira, Perego, Oliveira, & Converti, 2011); Inulin, especially long-chain inulin can form gels that consist of small crystallites with rheological properties that resemble that of fat, which turn out to be a good fat replacement in a water-continuous system with low dosage added(lmeson, 2010). However, at higher level of 2% and/or 3% of inulin added to the fat-free yogurt showed an increase of syneresis (Guven, Yasar, Karaca, & Hayaloglu, 2005). The results were mixed for yogurt sensory properties and pH decreasing rate during the yogurt fermentation (Aryana et al., 2007).

3.3 Vitamin D

Vitamin D is a derived from steroid. It is an important fat-soluble vitamin, which could hardly be found in fruits and vegetables, but it is rich in fish. Our skin is the major organ that synthesize the vitamin D when exposes to sunlight. The RDA is 400IU per day (NIH, 2019). Due to some reasons such as lacking outdoor activities, vegetarian diet,

high latitude habitat, the prevalence of vitamin D deficiency can be high (Parva et al., 2018). The vitamin D mainly participates in the serum calcium homeostasis, phosphorus homeostasis, cell differentiation, proliferation and growth, as well as muscle function. Meanwhile, being deficient in vitamin D in a long term may linked to autoimmune disorders, diabetes, certain types of cancer and CVD (Sareen et al., 2016). A birth-cohort study showed that children who receive plenty vitamin D supplement in the first year of life had a significantly lower risk of developing type I diabetes after entering the adulthood (Hyppönen, Läärä, Reunanen, Järvelin, & Virtanen, 2001). A recent study also shows that the combination of the vitamin D with probiotics therapy could significantly improve the health parameters for women with polycystic ovary syndrome (Ostadmohammadi, Jamilian, Bahmani, & Asemi, 2019).

Since the chemical properties of vitamin D is stable, it was added to many foods and beverages such as orange juice, milk as daily supplement to prevent the vitamin D deficiency for general population. Vitamin D_2 has comparable effect in increasing the blood 25-OH D (an active form of vitamin D) level as vitamin D_3 (Holick & Chen, 2008).

3.4 Calcium

Calcium is a major mineral in human nutrition. It plays an important physiological role on human health including bone mineralization and some other minor but important functions, including muscle contraction, enzyme activation and cellular processes (Sareen et al., 2016), etc. The RDA suggests 1000mg per day for general populations. Dairy products, vegetable from Cruciferae and some seafood are rich in calcium. The absorption can be enhanced by taking food with vitamin D, sugars or protein (Sareen et

al., 2016). In the gel-forming process, the divalent calcium cation acts as a bridge to help attracting the gelling molecules and forming linkages (Lapasin & Princl, 1995).

Chapter 4 Whey Proteins

4.1 Introduction of Whey Proteins

Milk proteins can be divided into two major groups: casein proteins and whey proteins. The casein/whey proteins ratio greatly varies in different animal species (Fox, McSweeney, O'Mahony, & Uniache-Lowe, 2015). In bovine milk, the whey proteins only account for 20% of total milk proteins. The fresh whey can be obtained from the cheese making process after the milk coagulates and further processed to whey protein concentration (25%-80% protein), whey protein isolate (90% and higher protein) and whey protein hydrolysate. Whey proteins are mainly comprised of four major proteins, including β-lactoglobulin (β-lg), α-lactalbumin(α-la), immunoglobulin (IG) and bovine serum albumin (BSA) as well as some minor types, including Lactoferrin (LF) and lysozyme (Santos, da Costa, & Garcia-Rojas, 2018). The relative proportion of those proteins varies in different animal species and lactation stage (Fox et al., 2015).

Since the whey protein is a by-product from cheese making, it is eco-friendly to recycle the valuable source and take advantages of it (Santos et al., 2018). Whey proteins are highly nutritious and at the meantime present functional properties, including gelatinization, emulsification and water holding capacity (Ju & Kilara, 1998).

4.2 Nutritional Value and Health Benefits

Whey proteins are complete proteins with high biological value of 104 (U.S Dairy Export Council, 1999). Unlike casein, it can be easily digested and quickly absorbed.

In bovine milk, β-lg accounts for 50-60% of total whey protein. There are total 11 genetic variants of β-lg from different animal species with different amino acids on certain sites and variants A and B are the most common types in bovine milk and besides, not all mammals have β-lg in their milk, for example, human milk does not contain this type of protein (Fox et al., 2015). β-lg is rich in branched chained amino acids (BCAA), which is well known as a sports supplement for few decades. Negro *et al.* summarized that the BCAA can support the muscle during/after an intensive exercise by reducing the muscle soreness; decreasing the protein degradation and positively regulating immune response for the tissue inflammation (Negro, Giardina, Marzani, & Marzatico, 2008). On the other hand, compare to ovine β-lg, the bovine β-lg was found to be more resistant to peptic digestion, which makes it becomes a potential allergens especially to children (El-Zahar et al., 2005).

The α-la accounts for 4% of bovine milk proteins but 25% for human milk protein. It is highly bioactive and can acts as a modifier to certain enzyme that could affect the lactose biosynthesis (Fox et al., 2015). The α-la can be modified and transformed into an α-la/oleic acid complex that could induce the apoptosis of cancer cells through cytotoxic activity (Jenny Pettersson-Kastberg et al., 2009; J. Pettersson-Kastberg et al., 2009; M. Svensson et al., 2003)

The Immunoglobulins are associated with the building of passive immunization for the neonates, thereby the concentration of Ig is depending on the stage of lactation, which could range from 3% to 10% of total protein in bovine milk (Fox et al., 2015).

LF is a glycoprotein. It has a very high affinity for iron as well as several other several physiological functions, thereby makes the LF crucial for the neonate's gastrointestinal health. The LF can eliminate bacteria by sequestering the iron so that bacteria cannot get access to the essential iron for the metabolism (Wakabayashi, Yamauchi, & Takase, 2008). Other important functions include but not limited to antiviral effects, building healthy microflora, immunoregulation and growth factors (Lonnerdal, 2013).

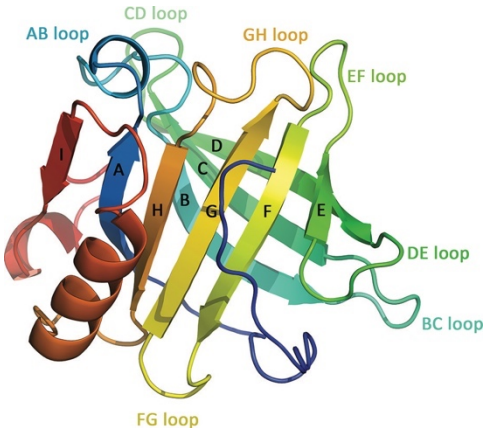
4.3 The Chemical Structure of β-Lactoglobulin

β-lg is categorized as a lipocalin from its chemical structure analysis, so it is believed to have the ability to bind those hydrophobic and chemically sensitive molecules, such as retinol, and deliver from mother to the young (Papiz et al., 1986).

In the primary structure of a β-lg monomer, there are 162 amino acid residues with 2 mol of cystine and 1 mol of cysteine, which means that there are two disulphide bridges from the cystine and one free thiol group from the cysteine (deWit & Klarenbeek, 1984). In the secondary structure, it has 3 α-helix, 9 β-sheet (labelled from A to I in the **Picture 1** from Crowther *et al*.) and some unordered structure including β-hairpins and β-turn, which comprise the loops that successively connect all β-strands from head to the end (Fox et al., 2015).

In the tertiary structure, β-lg follows the general folding pattern of kernel lipocalin, with a major calyx shape subunit that consists of 8 antiparallel β-sheets (A to H), thereby

forming a conical cavity that internally encloses the functional ligand-binding site in it (Flower, 1996). There is a 3-turned α-helix lies alongside the outer surface of the calyx that near G and H; also, the α-helix links to the ninth β-sheet I, which is flanking the A. The strand I is also associated with the formation of the dimer by interacting with the same strand from another monomer in an antiparallel align (Kontopidis, Holt, & Sawyer, 2004). The free thiol group from the residue Cys121 is beneath the α-helix and situated outward on the H.



Picture 1: 3D Structure of β-lactoglobulin (Crowther, Jameson, Hodgkinson, & Dobson, 2016)

4.4 Polymerized Whey Protein

The gelling function of the whey protein isolate is mainly achieved by the β-lg and the denaturation of β-lg can be altered by many factors including the change of pH, WPI solution concentration, ion concentration and temperature (Xiong, Dawson, & Wan, 1998). The PWP in this study was prepared by several steps:

1. Dissolve the whey protein isolate in deionized water as 10% WPI solution at room temperature with constant stir, store in the cold room overnight and let the foam to set.
2. Adjust the solution pH to 7.0
3. Heat the solution to 85℃ and hold for 30 mins and cool down immediately in the ice water to get the PWP solution.

Denaturation temperatures for major proteins in whey (deWit & Klarenbeek, 1984):

β-lactoglobulin	78℃
α-lactalbumin	62℃
immunoglobulin	64℃
bovine serum albumin	72℃

At the native state, the thiol groups from cysteine residues in the center of the calyx are undergoing redox reactions that form the disulphide bridge and become cystine internally, thereby stabilize the structure of the β-lactoglobulin (B. Y. Qin et al., 1998).

When the pH is lower than 3.5, the EF loop is shut, and the β-lg exists as a monomer; between pH 3.5-5.2, the β-lg exists as octamer. When pH is around 5.5-7.5, the β-lg exist as a dimer (Fox et al., 2015). When the pH is close to 7, the β-lg undergoes Tanford Transition, of which is a process of reversible conformational change. At this point, the EF loop is opened and the internal cavity is revealed allowing the ligand to get access to the binding site (Kontopidis et al., 2004).

At this stage, if the WPI solution is heated to 70℃ and above, the disulphide bridges are broken and reform randomly with any other functional groups of other molecules. Meanwhile, the β-lg become unstable and unfolded, which results in a larger volume of

the molecule and as the molecules aggregate, the viscosity of the WPI solution increases (B. Qin et al., 1998). Since the pH has previously adjusted to 7, which is above the isoelectric point of β-lg, the type of interactions is very limited because of the repulsive force. However, since the thiol groups also present a pH-dependent activity that the same with the Tanford Transition (Kontopidis et al., 2004), the disulphide exchange reactions are not affected. Most of the disulfide exchange interactions have done at this moment (Vardhanabhuti & Foegeding, 1999).

When this pre-heated PWP is applied to the yogurt, it forms the acid-induced cold-set gels as the pH drops during the fermentation. As the acidity increases, the pH is reaching the whey protein isoelectric point. As a result, the repulsive forces reduced, which allows β-lg molecules co-aggregate with themselves to form disulphide bridges and form fine smooth individual strands, or interact with other hydrocolloid behaved like a gel network, which results in an improved texture (Walsh, Ross, Hendricks, & Guo, 2010).

Chapter 5 Food Stabilizers and Gums

5.1 Introduction of Stabilizers and Gums

The stabilizers and gums are colloidal substances, usually carbohydrate polymers or a protein that fully hydrate in water and form a macromolecule network to modify the rheology of the system from two major aspects: viscosity and texture (Imeson, 2010).

The functions of the stabilizer and gums can be summarized as ① Thickening effect ② Gelling properties ③ Emulsification ④ Forming films and coatings ⑤ Fat replacer (Milani & Maleki, 2012). In addition to the functions, certain gums, such as pectin and Arabic gum, which also belong to soluble fiber, may have some potential health-promoting effects, including cholesterol reduction and weight loss (Imeson, 2010).

With regard to the classification, they are commonly grouped by raw material origin. **Diagram 2** shows the source and functions of several food stabilizers and gums.

Origin	**Stabilizers and gums**	**Major function**
Seed gums	Guar gum	Thickener
	Locust bean gum	Thickener
Fermentation products	Xanthan gum	Thickener
Plant exudates	Pectin	Gelling agent
	Starches	Thickener and gelling agents
Protein	Gelatin	Gelling agent
	PWP	Gelling agent and emulsifier

Diagram 2. The Origin and Major Function of Stabilizers and Gums (Imeson, 2010)

5.2 Locust Bean Gum

There are more details about the stabilizers and gums that had been used in the coconut yogurt in this study.

Locust bean gum (LBG) is extracted from the fruit of the carob tree, and the main structure is galactomannans, they are composed of a main linear (1→4)-β-D-mannan chains with some terminal D-galactose unit that linked to the main chain through (1→6)-

α-glycosidic bonds to the 4,6-mannose units(He et al., 2017). The guar gum, tara gum and LBG are distinguished by their mannose-galactose ratio, of which the LBG is about 3.5:1. Those galactose side units prevent too much cohesion formed between the polymer backbones. As a result, locust bean gum only increases the viscosity instead of forming gel (lmeson, 2010). The thickening ability of LBG is mainly determined by the distribution and galactose content, also influenced by the length of the galactomannan and the interaction with other stabilizer, such as xanthan gum (Khouryieh, Puli, Williams, & Aramouni, 2015). In addition, the emulsification ability can be greatly promoted by combining with modified starch and/or proteins (lmeson, 2010).

5.3 Starch

Starch is one of the most important hydrocolloids in food industry. It includes two types of components, the more linear shaped amylose (more α-1, 4 linkage D-glucopyranose) and the more branched amylopectin (higher α-1,6 linkage D-glucopyranose). When heated, the starch molecules will swell and hold water molecules and form gel. Study shows that when combine the LBG and corn starch in the solution, the viscosity and water-holding ability will increase significantly, whereas the gelatinization temperature decreases (Alloncle, Lefebvre, Llamas, & Doublier, 1989). The mechanism was elucidated by Doublier *et al.* that the swollen particles (dispersed phase) of amylopectin are suspended in a continuous phase that consists of the soluble amylose macromolecules, and this system is called starch pastes. When the galactomannan molecules are dispersed within the continuous phase, the dispersed phase

that accessible to the galactomannan is decreased, which resulting in a higher viscosity (Doublier, 1987).

5.4 Pectin

The native pectin is extracted from citrus peel and apple pomace and it belongs to polysaccharide that exists in 3 forms, including homogalacturonan (HGA), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). They are considered as dietary fibers. When they are further processed to commercial pectin, the latter two forms are largely removed and the linear homopolymers of 1,4-α-D-galacturonic acid become the primary component (lmeson, 2010). In the polymer HGA, some of the carboxyl groups of the uronic acids were naturally esterified with methyl group. On the other hand, the carboxyl groups can also be artificially methyl-esterified or de-esterified, which yield pectin with different ratio of methyl esterified galacturonic acid. This ratio is called the degree of methyl esterification (DE). The low methoxyl (LM) pectin has a DE ranges from 20~45% and the high methoxyl (HM) pectin has a DE of 60~75%. The LM can be further amidated.

In food application, pectin shows the best stability at the pH 3.5-4.0 and the temperature below 70℃. The HM and LM pectin require different conditions to develop their gelling property. The HM pectin need a low pH and a high sugar content; therefore, HM is a perfect gelling agent for gam making. Whereas the LM pectin gels at a wide range of pH, also does not need high sugar content, nevertheless, the presence of a lower amount of sugar may decrease syneresis and improve the firmness of the gels

(Pornsak Sriamornsak, 2003). The pectin also requires the presence of cations, such as calcium to form gels by binding the homogalacturonic region (Grant, Morris, Rees, Smith, & Thom, 1973). LM pectin has been widely applied to products of yogurt and beverages.

MANUSCRIPT

CHEMICAL PROPERTIES AND PROBIOTICS SURVIVABILITY OF SYMBIOTIC COCONUT YOGURT USING POLYMERIZED WHEY PROTEIN AS A GELATION AGENT

Jinglin Lu

Department of Nutrition and Food Sciences,
University of Vermont

Abstract

Food products from coconut fruits are delicious and nutritious; the coconut water could be directly used as a sports drink; whereas the meat can be processed to coconut milk, coconut puree and other products that are rich in medium fatty acids that can be burnt quickly to provide energy to the body. In recent years, products that incorporated the probiotics with coconut have been developed, however the texture and nutritional qualities were not satisfied.

The purpose of the study was to create a functional coconut yogurt using polymerized whey protein (PWP) as a gelation agent to achieve a better mouthfeel and also increase the protein content of the final product. In the preliminary studies, different ratios of PWP/stabilizer system were tested to optimize the formulation. After the formula was finalized, three groups including control yogurt, PWP fortified yogurt and Ca/vitamin D fortified yogurt of 3 trials were made for further analysis.

The chemical composition of the coconut yogurt including protein, fat, minerals, total solids and ash were analyzed. A sensory evaluation was also conducted to compare the lab samples from each group with the commercial samples. Result showed that there was no difference of yogurt properties within three lab groups except for the firmness. However, the lab groups had a significantly ($P<0.05$) higher score for most properties than commercial group.

pH values, viscosity and probiotics survivability were also analyzed weekly during the 8 weeks of storage. The pH significantly increased ($P<0.05$) for all groups, whereas the viscosity showed a slow but significant decrease in all groups ($P<0.05$) with the same decreasing rate ($P>0.05$). At the last week of study, only *Lactobacillus paracasei* were above the therapeutic level in all three groups. *Lactobacillus acidophilus* did not reach that level at the first week of storage. The difference of survivability among three groups were all significant different ($P<0.05$).

Introduction

Symbiotic coconut yogurt is a fermented food that combines the coconut meat, coconut water, probiotics and prebiotics in the final product. The health benefits from probiotics have been extensively studied, including the inhibition of the pathogenic bacteria in intestine, immune system promotion, improvement of irritable bowel syndrome and cancer prevention (Kailasapathy & Chin, 2000). Prebiotics have a synergistic effect when consumed with probiotics by improving the probiotics viability (Donkor, Nilmini, Stolic, Vasiljevic, & Shah, 2007). The medium chain fatty acids (MCFAs) from coconut meat are the most noticeable character of coconut (Dayrit, 2014). Studies indicated that MCT might also help to prevent the body fat accumulation (DeLany, Windhauser, Champagne, & Bray, 2000) and reduce the risks of coronary heart disease (CHD) by improving the cholesterol ratio (Pehowich, Gomes, & Barnes, 2000). Coconut water is traditionally used for electrolyte drink even intravenous hydration fluid as it has a balanced mineral contents to replenish body fluid and keep the electrolyte homeostasis (Pummer, Heil, Maleck, & Petroianu, 2001). The coconut fruit also contains polyphenols as well as antioxidant protein fractions that could reduce the oxidative stress by neutralizing the free radicals (Yan Li et al., 2018; Tan et al., 2014).

Since coconut is tasty and rich in nutrients, several fermented coconut milk drinks/yogurts have been developed. However, the sensory properties of these products need to be improved (Yuliana, Rangga, & Rakhmiati, 2010). The reason is, unlike dairy, the coconut meat and coconut water are lack of protein, leading to a weak gel or no gel formation, also causing wheying off.

In this study, PWP was used as a co-gelation agent to formulate a set type coconut yogurt with a good texture and improved protein level. The purpose of this study is to determine whether the PWP can act as an appropriate gelation agent in the coconut yogurt matrix

Materials and Methods

Organic coconut puree (Nutiva Inc., CA), ZICO natural coconut water (ZICO Beverage LLC., NY), organic cane sugar (Wholesome Sweeteners Inc., TX, USA) were purchased from Amazon.com. GRINDSTED®Yogurt 6895 Stabilizer System was provided by Danisco. Calcium citrate tribasic tetrahydrate 98%, Vitamin D_2 (ACROS Organics™, NJ), was ordered from Fisher Scientific™. Orafti® HPX inulin was provided by Beneo-ORAFTI, Belgium. Pectin (GENU®type LM-106 AS-YA, CPKelco, Denmark) was provided by CPKelco. The starter culture (YoFlex® YF-L02 DA, Chr Hasen, WI, USA) was provided by Chr Hansen. Whey protein isolate (WPI 940) was provided by HILMAR, CA.

Preparation of Polymerized Whey Protein Isolation

Preparation of 10% (w/v) whey protein (WP) solution, 10.87g of WPI (92% whey protein) was completely dissolved in 100ml of deionized water. The solution was kept at 4℃ overnight to be rehydrated. The WP solution was later adjusted to pH 7.0 with 2M NaOH at room temperature, then heated in a water bath at 85℃ for 30 minutes, which allowed the protein to be denatured and polymerized. Then, the polymerized whey

protein (PWP) solution was cooled immediately down to 4℃ for storage.

Preparation of Starter Culture Solution

Two grams of starter culture was added to 99ml of sterile peptone buffer solution and mixed well. The inoculation rate was 0.02% following the manufacture's recommendations.

Optimization of Formulation

300ml coconut water was mixed with 50 grams of coconut puree with Stabilizer System (SS) and PWP. Then the mix was heated to 55 ℃. Then 0.5% pectin, 4% of sucrose and 1% of inulin were added and mixed with 100ml coconut water and then heated to 80℃. The solution was allowed to cool down to 55℃, then poured into the coconut water/coconut puree mixture to form the yogurt base. It was then homogenized for 3 minutes by a blender. Finally, the base was heated to 80 ℃ and held for 5 minutes with constant stirring, then cooled down to 42℃. The base was then ready to be inoculated with the starter culture. Four groups with different ratios of Stabilizer System (SS) and PWP were made to optimize the formulation: 1.0%SS+0.4%PWP, 1.0%SS+0.8%PWP, 2.0%SS+0.4%PWP and 2.0%SS+0.8%PWP. The pH and viscosity were measured during fermentation. When the pH fell into 4.5~4.6, the fermentation was stopped and cooled down to 4 °C. All the values were calculated from 3 replications drawn from each group.

After the formulation and incubation time were determined, three groups (control yogurt, PWP fortified yogurt and Ca/vitamin D fortified yogurt) of products were prepared, three trials were made for each group to determine the functionality and any significant difference of PWP in the product by weekly measuring the pH and viscosity, probiotics survivability as well as the chemical composition. The control yogurt was prepared using 2.0% SS; the PWP fortified yogurt with 2.0% SS + 0.8% PWP; the Ca/vitamin D fortified yogurt was prepared with additional calcium citrate tribasic tetrahydrate (>98%) and vitamin D_2 (97%) at a concentration of 4.74g per Liter and 400 IU per liter, respectively. The samples were incubated at 40.5℃ for 3.5-4.0 hours until the pH reached 4.6. Then, all the samples were refrigerated at 4℃ and stored for 8 weeks.

Chemical Analysis

The samples from each group were analyzed for chemical composition including total solids, ash, fat, crude protein and minerals, using AOAC methods (AOAC International, 20th Edition, 2016). The pH and viscosity were measured weekly, using a pH meter (Fisherbrand™ by Thermo Fisher Scientific) and a viscometer (Brookfield Engineering Laboratories, Inc., Model LVDV-I), respectively. The viscosity values were expressed in mPas and set for 2 mins measurement period at 100 rpm with model LV #4 spindle at room temperature 19±1.5 °C.

Probiotics Survivability and Shelf Life Determination

Samples were taken from each group weekly for probiotics enumeration. The samples were processed by a series of dilutions. The pour plate method was used to carry out the probiotic enumeration. The enumeration for *L. acidophilus* and *L. paracasei* was carried out according to the methods of Chr Hansen (2019).

For the *L. paracasei* enumeration, the MRS agar was used. The plates were incubated at 20-22 °C for 6 days. The colonies of this species appeared to be large, smooth bodied, white in color with a round edge and shiny surface.

For the *L. acidophilus* enumeration, additional inhibitors including clindamycin and ciprofloxacin were added to the MRS medium to suppress the growth of other species. The samples were anaerobically incubated at 37 °C in a GasPak Plus™ jar (BBL) with 2 AnaeroGen™ 3.5L packs with anaerobic indicator (Thermo Scientific Oxoid Ltd, UK) for 3 days. The colonies appeared to be small in size, cream colored and irregularly star shaped.

For the bifidobacteria enumeration, the BSM (Bifidus Selective Medium by MilliporeSigma, Merck KGaA, Darmstadt, Germany) was used for the isolation and enumeration. The samples were anaerobically incubated at 37 °C for 3 days. The colonies were recognized as purple in color, small, and round with a smooth surface.

Statistical Analysis

All analyses were performed using JMP statistical software, with $\alpha=0.05$ for all tests. For probiotics, pH and viscosity, the Fit Model platform of JMP was applied to perform

an analysis of covariance with a null hypothesis that there is no interaction between the trend across weeks and groups. For the sensory evaluation, the Fit Y by X platform was used to perform a one-way ANOVA on each property of the sensory evaluation. The null hypothesis was that there was no difference between the groups for each property.

Coliform and Yeast/Mold Counts

Samples were randomly selected from trials of each group. The coliform and yeast/mold were tested twice during the storage at week one and week five. The Yeast and Mold Petrifilm™ (3M Petrifilm™) was used for the yeast/mold counting and incubated at 21℃ for 6 days; The Coliform Petrifilm™ (3M Petrifilm™) was used for the coliform counts and incubated at 37℃ for 48 hours.

Sensory Evaluation

A product-oriented sensory evaluation was carried out by a panel of 11 individuals. Students and faculty on campus were randomly chosen to participate. The panelists were briefly trained by reading handouts with related information about the coconut yogurt to get a reliable judgement. They were also provided with a consent form and an allergen information form before evaluation. They were served 3 freshly made samples from each lab group and one commercial sample. Every sample was approximately 60g and contained in a transparent plastic sample cup at 4 °C. Each sample came with a separate survey form. The survey included the coconut yogurt properties including texture, appearance and flavor. The panelists were asked to evaluate the samples and score all of

the yogurt properties using a hedonic scale scoring system that ranged from 1 to 5, where 1 represents “extremely unsatisfied” and 5 extremely satisfied (Chandan & Kilara, 2013; Watts, Ylimaki, Jeffery, & Elias, 1989). All of the panelists participated in the sample tasting and individually completed the survey with no verbal communication with other panelists during the evaluation process. The sensory evaluation was reviewed and approved by the University of Vermont Institutional Review Board.

Results and Discussion

Optimization of Formulation

With respect to the pH among the four formulations, the 2.0%SS+0.8%PWP dropped faster than other 2 groups (**Figure 1**). This may attribute to the stimulatory effect from the carbohydrate content (the main chemical composition of stabilizer system).

Figure 2 showed that the samples with 2.0%SS have high viscosity. This may indicate that the SS can improve the “fuller” mouthfeel of the yogurt. However, there was no significant difference in viscosity between the sample with 2.0%SS+0.4%PWP and the one fortified with 2.0%SS+0.8%PWP ($p=0.1424$). The yogurt texture with 0.8% PWP turned out to be firm; it appeared a natural coconut white color with a shining jelly surface. The coconut yogurt also had a reasonable sweetness and sourness.

Taking the protein level into consideration, 2.0%SS+0.8%PWP was selected as the final formulation.

Chemical Composition

The gross chemical composition of the symbiotic coconut yogurt samples was shown in **Table 2**. The crude protein content was converted from the nitrogen content by converting factor, which is 5.30 and 6.38 for coconut and whey protein, respectively (Jones, 1941). The total carbohydrate content was calculated from the total solids minus the contents of protein, fat and ash, which about 13g per 100g coconut yogurt including added 4% sugar, 1% inulin and 0.5% pectin from the formula.

Coliform, Yeast and Mold

Coliforms were found in two groups from trial 1 and two groups from trial 3, which ranges from $9.5*10^2$ CFU/g to $1.3*10^4$ CFU/g. Similar results were found at week 5, the coliforms were found in the same samples that were previously contaminated, with a slight increase in counts. This may be due to uneven heat treatment and/or the unintended contact of a contaminated glove.

Probiotics Survivability and Shelf Life

- ***Bifidobacterium***

There was no significant difference in the decreasing rate for all groups during the 8-week storage ($p=0.9227$). Every group had a significant decrease for the population of *Bifidobacterium* during the storage ($p<0.0001$), but the difference was significant among 3 groups ($p<0.0001$) (see **Table 3**).

The packaging may account for the decreasing survivability of all groups, since the containers are not sealed. The oxygen dissolved in the yogurt is detrimental to those obligate anaerobic cultures.

As shown in **Figure 7**, the control sample had the highest pH during the storage period. Also, the control yogurt also had the best viability (see **Figure 3)**, which may indicate that a lower pH environment is adverse to the *Bifidobacterium*. This observation was consistent with a study which demonstrated that less than half of the strains from different species of *Bifidobacterium* can survive in the pH from 3.7 to 4.3 (Lankaputhra, Shah, & Britz, 1996).

- ***L. acidophilus* (LA)**

There was a significant difference for the decreasing rate among groups during the 8-week storage ($p=0.0403$). LA counts decreased significantly number during the 8-week storage ($p<0.0001$). (see **Table 3**)

The rapid loss of *L. acidophilus* in yogurt was observed in many studies, the survivability of LA may be determined by the combination of culture species, the fermenting substrate (plant-based and/or dairy), storage temperature, and pH, etc.,

A strain-specific study showed that the La05 strain was inhibited by the skim milk cell-free supernatants of *Bifidobacterium* Bb-12 strain but no inhibition from the same strain's MRS cell-free supernatants; whereas none of LB strains in that study showed an inhibiting effect on the La05 strain (Vinderola, Mocchiutti, & Reinheimer, 2002). This may indicate that certain strains of *Bifidobacterium* contained in the starter culture may be competitive or antagonistic to the LA.

It was reported that LA showed the best growth and had a good survivability during the 16 days storage in 5℃ (Yuliana, Rangga, & Rakhmiati, 2010). In that experiment, the coconut milk was allowed to ferment for 24 hours and as a result, the LA became the predominant bacteria in the fermented coconut milk. This may indicate that in a certain fermenting substrate, LA may need much longer time to grow and reproduce. However, this assumption needs to be verified because in that study the survivability was only tested for about two weeks.

The low count of LA may also be correlated to the media that was used for isolation and enumeration. Dave & Shah (1995) found that the enumeration varies greatly depending on the different medium used. The enumerations were also different when using the same medium at a different pH.

L. acidophilus survived better in the Ca/vitamin D fortified yogurt, which indicates that the calcium ions may have a positive effect on the survival of LA (see **Figure 4**). A study suggests that the calcium ion can induce the change of cell morphology of LA, which makes the cells become more adaptive to the environmental stress during the freezing process (Wright & Klaenhammer, 1981). This observation may imply that the calcium citrate that was added to the Ca/vitamin D fortified yogurt may have a similar effect on its survivability during the storage.

- ***L. paracasei***

There was no significant difference in LP population among the 3 groups during the 8-week storage (p=0.3330), which indicates that the number of LP is stable. There was no significant difference in the changing rate among groups (p=0.2596). *L. paracasei* is a

robust and stable probiotic, which shows a stable survivability in studies (Gokavi, Zhang, Huang, Zhao, & Guo, 2005).

Change in Viscosity During Storage

As seen in **Table 3**, the viscosity had decreased significantly during the 8 weeks of storage in all groups ($p<0.0001$) and the difference in viscosity was also significant among groups ($p<0.0001$). The Ca/vitamin D fortified yogurts had the highest viscosity value during the 8-week storage. However, there was no significant difference in the decreasing rate ($p=0.6635$).

The molar concentration of the calcium ions in the Ca/vitamin D fortified yogurt is about 28.5mmol/L, and 4mmol/L for the other two groups. According to a study of the relationship between the PWP and calcium cation, the shear stress was significantly increased ($p<0.05$) from 30.4kPa to 43.0kPa as the calcium level increased from 10mmol/L to 30mmol/L under the same shear rate (Barbut, 1995), this may help to explain the higher viscosity in the Ca/vitamin D fortified yogurt (see **Figure 6**). Meanwhile, the presence of calcium cations can help the LM pectin to form gel. Generally, the higher the level of calcium added, the firmer the gel formed, though the amidated LM pectin requires less calcium (Raj, Rubila, Jayabalan, & Ranganathan, 2012; Pornsak Sriamornsak, 2003).

The PWP could form a cold-set or acid-induced gel network by interacting with other molecules including polysaccharides, thereby fortify the gel structure (Walsh, Ross,

Hendricks & Guo, 2010) which results in a higher viscosity in the PWP fortified yogurt compared to the control yogurt.

Bifidobacteria and LA may slowly metabolize both starch and locust bean gum as the their carbon sources (De Vos et al., 2009). Since those polysaccharide molecules are a part of the gel network, once digested by the bacteria, the gel structure may be changed.

Change in pH During Storage

The pH values of all samples significantly increased during the 8-week storage ($p<0.0001$). However, the increasing rate was similar to the groups*week ($p=0.7761$). The pH was significantly different among groups ($p=0.0006$). The control yogurt had the highest pH, then the Ca/vitamin D fortified yogurt, and the PWP fortified yogurt was the lowest in pH value.

The *L. bulgaricus* can hydrolyze the casein protein into amino acids, which could subsequently stimulate ST to produce more acids during the fermentation (Moon & Reinbold, 1976). However, the hydrolysis effect on the coconut protein is unknown. If the coconut protein could be partially broken down into amino acids, since the second richest amino acids in mature coconut meat is the basic arginine (see **Table 1**), it is assumed that this may contribute to the slightly increased pH during the storage.

In the Ca/vitamin D fortified yogurt, the calcium citrate may combine the trace amount of citric acid from the coconut water to form a weak buffer system, which could stabilize the pH (Whittier, 1937; Yong et al., 2009). This may explain the difference between the PWP fortified yogurt and Ca/vitamin D fortified yogurt (see **Figure 7**).

Some LAB has been reported to possess the acid stress response system to deal with low acid environment by producing basic compounds (Guchte et al., 2002), these activities may be responsible for the increased pH during storage. The arginine deiminase pathway can be found in many LAB species to hydrolyze the arginine to CO_2 and NH_3. However, none of the species in the starter culture contains that gene (De Vos et al., 2009; Hwang & Lee, 2018). Meanwhile, the urease activity was detected in most *S. thermophilus* strains and this characteristic is associated with the breaking down of amino acids to NH_3, which may cause the delay of milk acidification in yogurt production (Mora et al., 2005; Tinson, Broome, Hillier, & Jago, 1982).

Moreover, some species including *L. brevis* and *B. infantis* possess the enzyme that can decarboxylate glutamic acid and they also have the genes that encode the function of amino acid antiport. These properties make it possible, even in resting cells, to transport a glutamic acid into the cytosol of the cell and convert it to a more alkaline γ-aminobutyrate (GABA) and release them to the environment under certain conditions, including a low pH and an adequate glutamate supply (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012; Higuchi, 1997; Sanders, 1998).

Sensory Properties of the Symbiotic Coconut Yogurt

- **Color**

Within groups: There was no significant difference between samples in the color, the average score over 4.00. The result indicates that the PWP had no negative effect on the color of the product.

Compared to commercial: Since the yellowish color can be observed, the commercial samples got a significant lower score of 2.81 for the color ($p=0.0007$).

- **Wheying off**

Within groups: There was no significant difference in wheying off between samples. The PWP fortified yogurt samples and Ca/vitamin D fortified yogurt samples got the highest score of 4.91. There was no wheying off observed during the 10 weeks of storage for all groups.

Compared to commercial: The commercial coconut yogurt got a significantly ($p<0.0001$) lower score of 3.54 than the symbiotics coconut yogurt because there was visible water separated from the yogurt body. The commercial samples were obtained from the grocery store, the yogurt structure may have been disrupted by shaking and/or moving during the transportation and consequently the syneresis may have occurred. On this point, the wheying off evaluation may not reflect the accurate result between the experimental groups and commercial group.

- **Firmness**

Within groups: The Ca/vitamin D fortified yogurt and PWP fortified yogurt got the highest score (4.09 and 4.00, respectively) on the firmness. There was no significant difference in firmness between these two groups, but the Ca/vitamin D fortified yogurt got a significantly higher score than the control yogurt (**Table 4**).

Compared to commercial: The commercial yogurt got a significantly lower score of 2.36 on the firmness ($p<0.0001$) as compared to the experimental yogurt groups.

The results indicated that the PWP helps to improve the firmness of the symbiotic coconut yogurt. Meanwhile, the additional calcium dication may also help to interact with the pectin and PWP to form a firmer gel by bridging the gum molecules (Ventura, Jammal, & Bianco-Peled, 2013).

- **Granule**

Within groups: The Ca/vitamin D fortified yogurt got a significantly lower score of 2.91 than the control, which scored 4.18. This may be due to the calcium addition. When the calcium ion is at a lower concentration (10mmol/L), it helps the gum molecules to aggregate and form a fine polymerized gel structure. On the other hand, a higher calcium concentration (≥30mmol/L) in the PWP hydrocolloidal system would result in a firmer gel but rougher structure with large protein strands (Barbut, 1995; Ju & Kilara, 1998).

Compared to commercial: The commercial yogurt got a significantly higher score of 4.45 than the Ca/vitamin D fortified yogurt.

Despite the significance, the commercial yogurt got the highest score on the granule aspect. The major ingredients in the commercial yogurt are coconut milk and water. The coconut milk is a smooth liquid and it was finely filtered from the grated coconut meat. However, in experimental groups, the whole coconut puree was used to produce the symbiotic coconut yogurt. Since the whole coconut puree retains everything from the mature coconut meat, including the insoluble dietary fiber, and this is the cause of the granulous texture of the symbiotic coconut yogurt.

- **Sourness**

Within groups: There was no significant difference in sourness among groups. The PWP fortified yogurt got the highest score of 3.64.

Compared to commercial: There was no significant difference between the symbiotic coconut yogurt and commercial one.

The result indicated that the starter culture performed well in the coconut yogurt and the yogurt contained an appropriate level of organic acid to give a balanced sourness to the products.

- **Sweetness**

Within groups: There was no significant difference in sweetness among all 3 groups. They were all scored over 4.00, which indicate that the sweetness is satisfactory.

Compared to commercial: The commercial yogurt got a significantly lower score of 2.73 in sweetness than the experimental coconut yogurt ($p=0.0002$). The results indicated that there was too much sugar added to the commercial yogurt, whereas only 4% of sugar was added to the symbiotic coconut yogurt.

- **Coconut flavor**

Within groups: There was no significant difference in coconut flavor among all groups. All groups were scored over 4.00, which indicated that the PWP does not bring any unpleasant or strange flavor as a gelation agent. The lab samples had a natural coconut taste.

Compared to commercial: The commercial samples got a significantly lower score of 2.72 as compared to the experimental samples ($p=0.0018$).

Conclusions

Results showed that the PWP could be a good gelation agent for the coconut yogurt. The yogurt texture and mouthfeel were improved by adding PWP; the protein content in the group with PWP was nearly doubled compared to the control. PWP also reduced the rate at which the viscosity decreased during storage. With regard to probiotic survivability, the *L. acidophilus* did not reach the therapeutic level, the *L. paracasei* was stable during the 8 weeks of storage, and the population of *Bifidobacterium* remained above the therapeutic level until the fourth week. Sensory evaluation data indicated that the symbiotic coconut yogurt samples were better than the commercial sample in terms of the texture, appearance, and flavor.

Figure 1. Effects of Fermentation Time on pH Value of the Coconut Yogurt

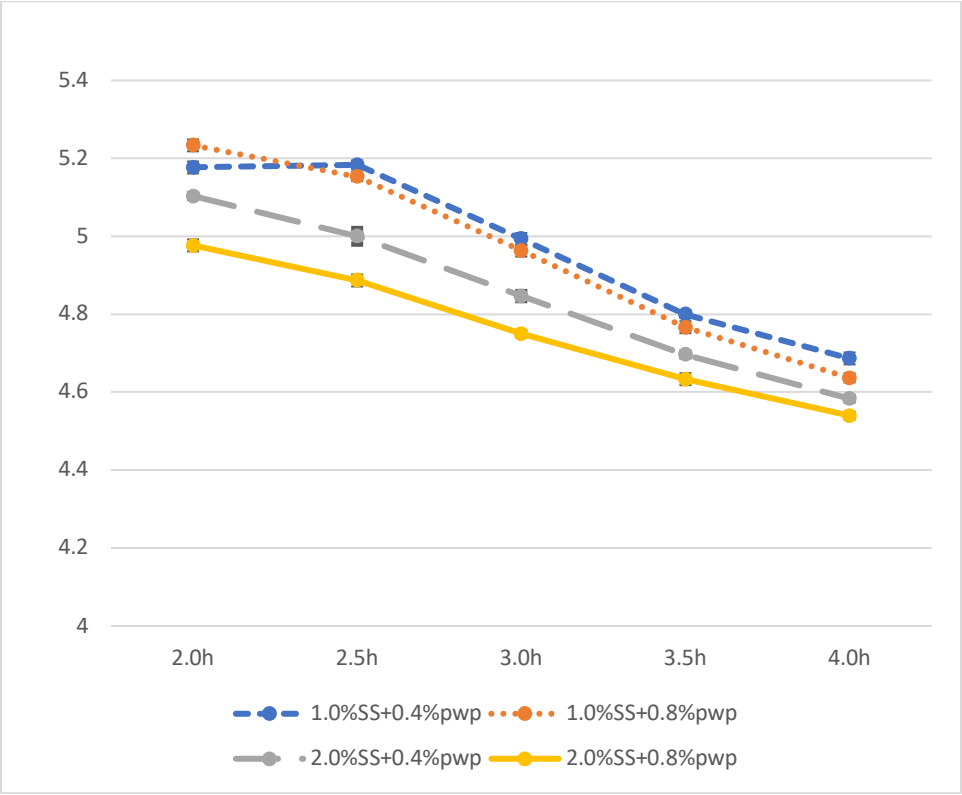


Figure 2. Effects of Different Formulations on Viscosity of the Coconut Yogurt

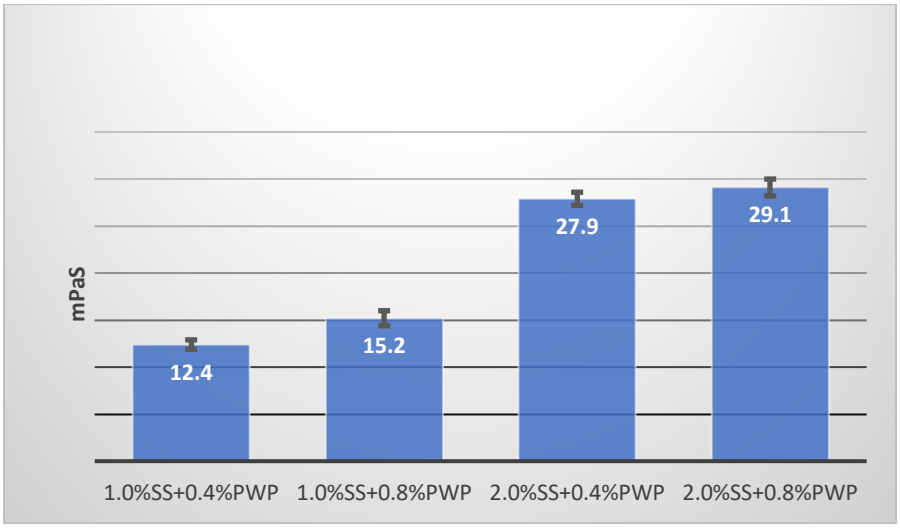


Table 2. Chemical Composition of the Control, PWP Fortified and Ca/Vd Fortified Coconut Yogurt

Nutrients	Control	PWP fortified	Ca/Vd Fortified
Total solids %	21.07 ± 0.0036	21.15 ± 0.0034	20.96 ± 0.0049
Ash %	0.62 ± 0.0012	0.65 ± 0.0021	0.61 ± 0.0015
Protein %	0.80 ± 0.03	1.51 ± 0.05	1.49 ± 0.00
Fat %	6.19 ± 0.40	5.64 ± 0.40	5.45 ± 0.0032
Minerals (mg/100g)			
C	16.33 ± 0.54	17.39 ± 0.55	111.23 ± 7.81
P	25.01 ± 1.73	25.34 ± 0.39	25.94 ± 1.17
K	207.46 ± 3.93	208.14 ± 7.82	212.42 ± 11.86
Mg	17.43 ± 0.77	17.38 ± 0.30	18.53 ± 3.07
Na	28.30 ± 2.20	35.59 ± 2.32	36.49 ± 3.07
Fe	0.38 ± 0.14	0.41 ± 0.15	0.49 ± 0.10

Table 3. Statistical Analysis of Changes in Probiotics, Viscosity and pH During 8 Weeks of Storage

Source of variation		P value	Significant (Yes/No)
Bifidobacterium	Group	<0.0001	Yes
	Week	<0.0001	Yes
	Group*Week	0.9227	No
L. acidophilus	Group	0.0001	Yes
	Week	<0.0001	Yes
	Group*Week	0.0403	Yes
L. paracasei	Group	<0.0001	Yes
	Week	0.3330	No
	Group*Week	0.2596	No
Viscosity	Group	<0.0001	Yes
	Week	<0.0001	Yes
	Group*Week	0.6635	No
pH	Group	0.0006	Yes
	Week	<0.0001	Yes
	Group*Week	0.7761	No

Figure 3. Survivability of *Bifidobacterium* in the Symbiotic Coconut Yogurt During Storage

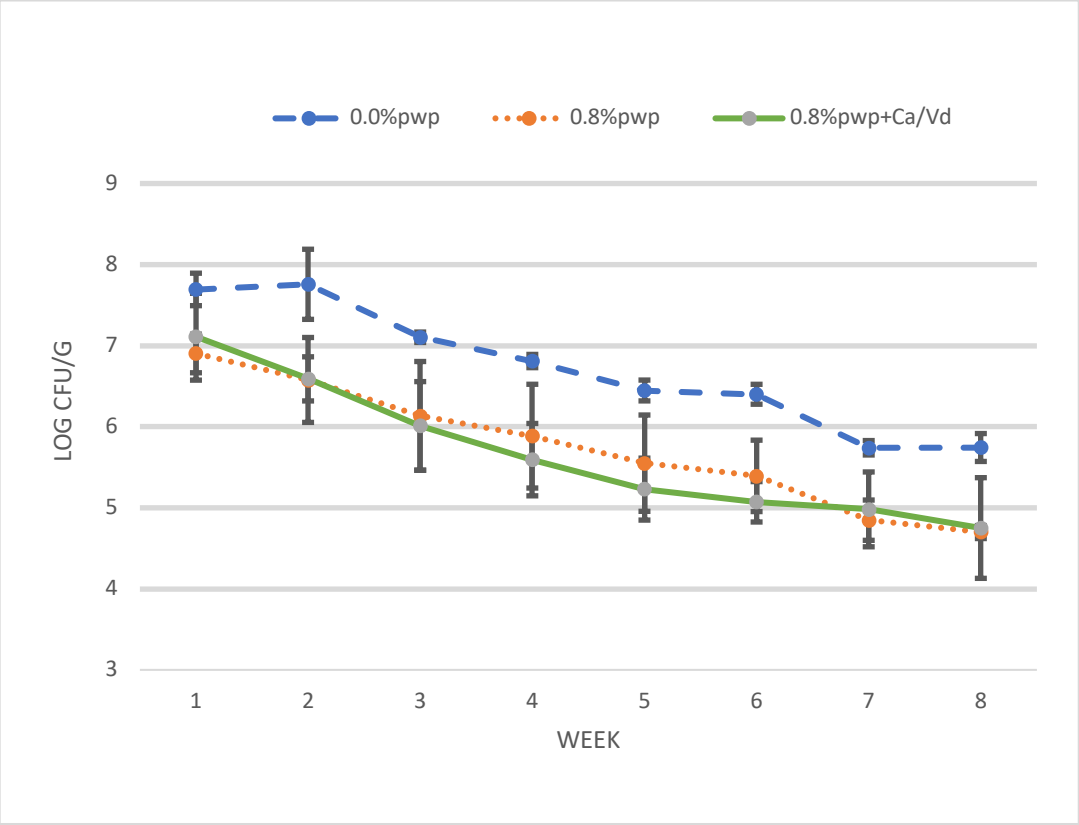


Figure 4. Survivability of *L. acidophilus* in the Symbiotic Coconut Yogurt During Storage

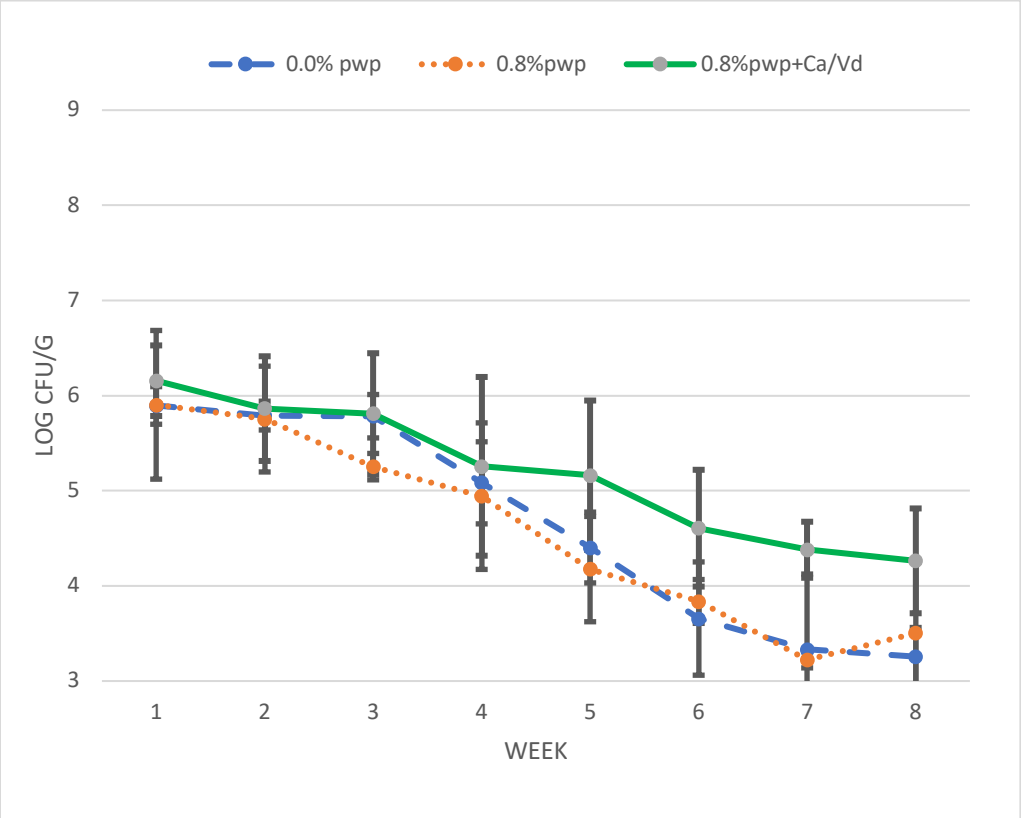


Figure 5. Survivability of *L. paracasei* in the Symbiotic Coconut Yogurt During Storage

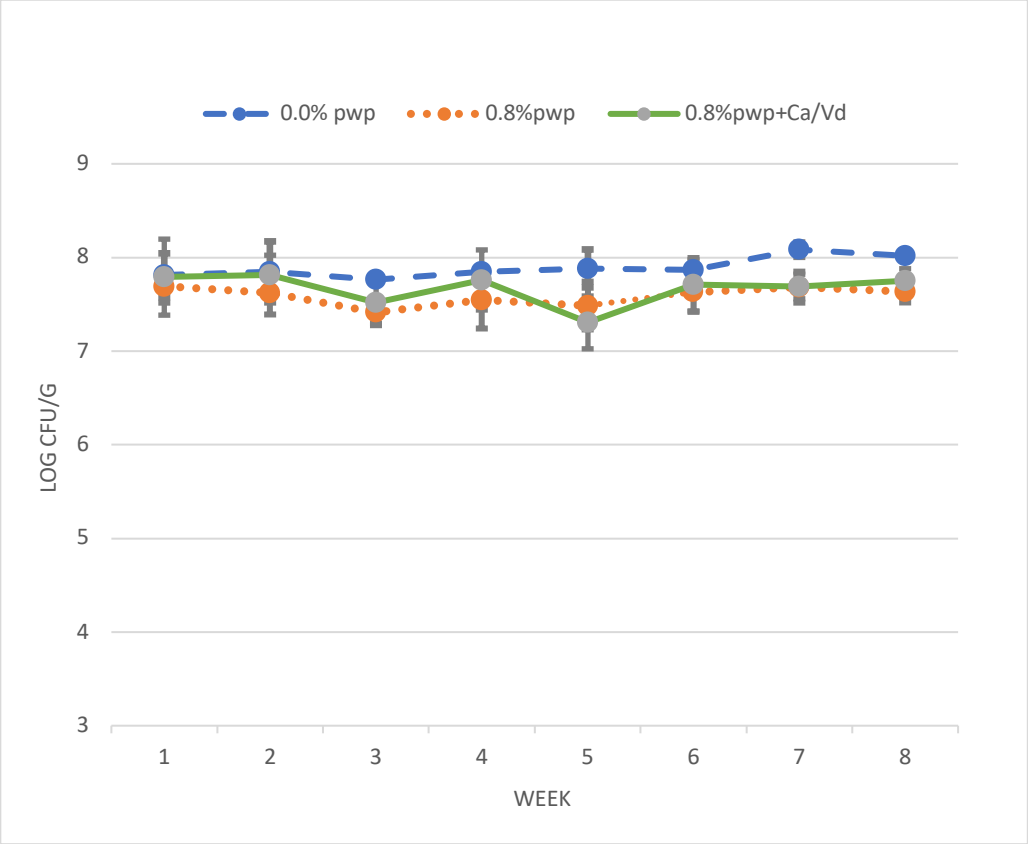


Figure 6. Changes in Viscosity of the Symbiotic Coconut Yogurt During Storage

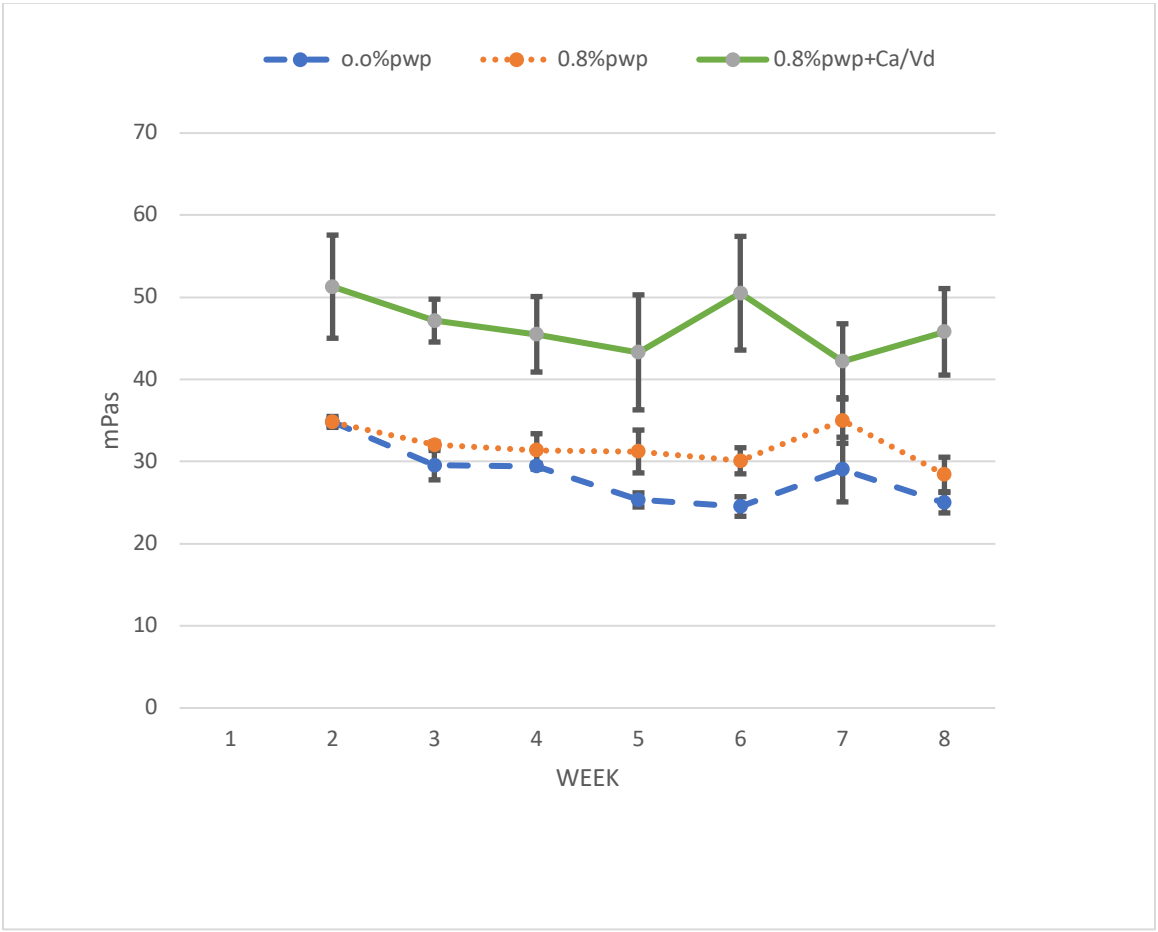


Figure 7. Changes in pH of the Symbiotic Coconut Yogurt During Storage

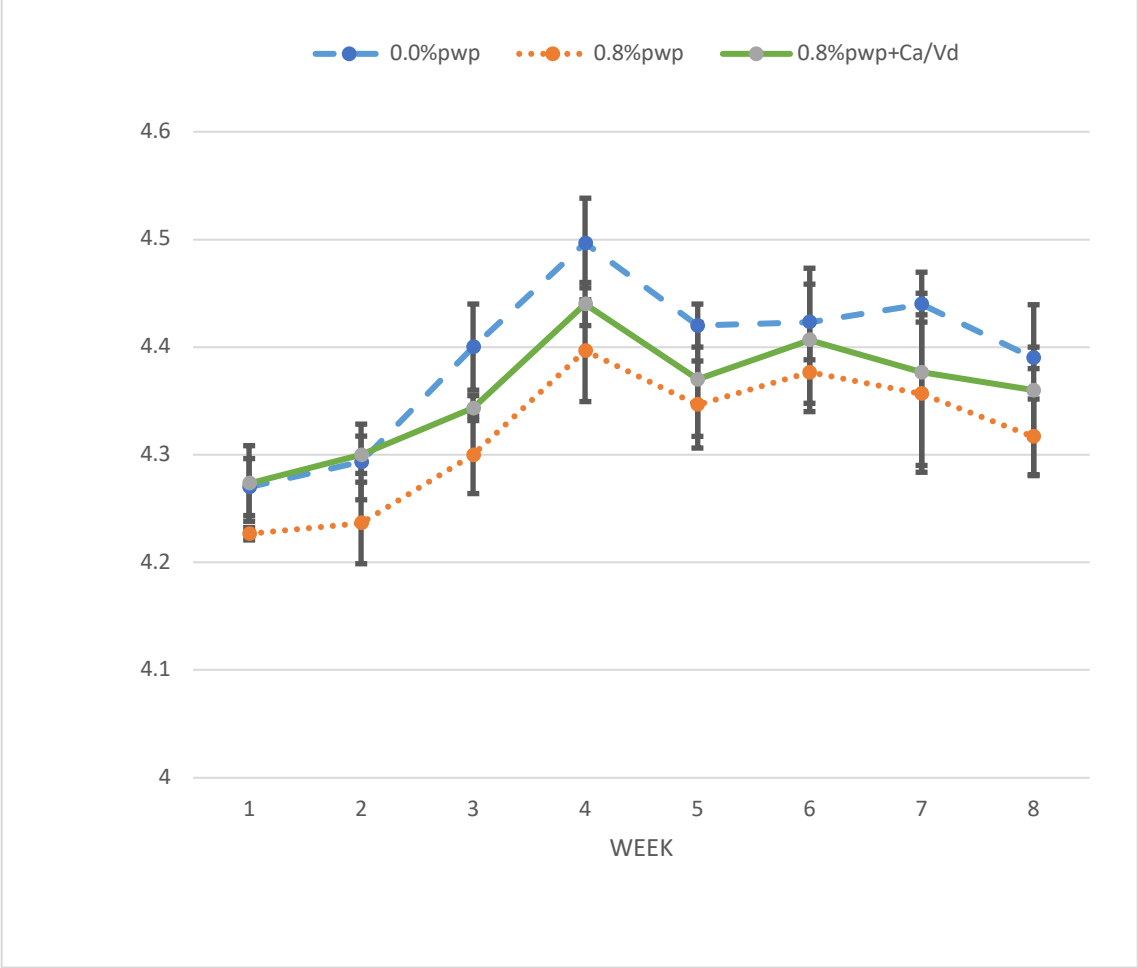


Table 4. Sensory Evaluation Result

Characters	Group	Connecting Letters	Mean value	Std Dev	p-value
Color	Control	A	4.18	0.40	0.0007
	Ca/Vd fortified	A	4.09	0.54	
	PWP fortified	A	4.00	0.63	
	Commercial	B	2.81	1.33	
Firmness	Ca/Vd fortified	A	4.09	0.83	<0.0001
	PWP fortified	A B	4.00	0.89	
	Control	B C	3.09	1.04	
	Commercial	C	2.36	0.67	
Wheying off	PWP fortified	A	4.91	0.30	<0.0001
	Ca/Vd fortified	A	4.91	0.30	
	Control	A	4.55	0.69	
	Commercial	B	3.54	1.03	
Granule	Commercial	A	4.45	0.52	0.0008
	Control	A	4.18	0.87	
	PWP fortified	A B	3.73	0.79	
	Ca/Vd fortified	B	2.91	1.14	
Sourness	PWP fortified	A	3.64	1.21	0.8213
	Control	A	3.27	1.49	
	Commercial	A	3.27	1.10	
	Ca/Vd fortified	A	3.18	0.98	
Sweetness	Control	A	4.09	0.54	0.0002
	PWP fortified	A	4.09	0.54	
	Ca/Vd fortified	A	4.00	0.63	
	Commercial	B	2.73	1.19	
Coconut flavor	Control	A	4.44	0.69	0.0018
	Ca/Vd fortified	A	4.27	1.27	
	PWP fortified	A	4.09	1.04	
	Commercial	B	2.72	1.27	

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