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Development Sangyod rice protein-based formula for cow milk allergic infant and its gut microbiota modulation

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ABSTRACT

Background: In Thailand, cow milk allergies are the leading food allergy in infants. Rice protein is one of the hypoallergenic food ingredients used as an alternative plant-based protein in nutritional products. Sangyod rice (*Oryza sativa*, L., var. *indica*), found in southern Thailand, has been reported to contain a high content of nutrients. Isomaltooligosaccharides (IMO) are accepted as prebiotics and are applicable as food ingredients to enhance the physiochemical quality of foods as a sweetener, and can also have physiological functions including the enhancement of gut microflora. The supplementation of formulas with prebiotics will support a mature immune system and intestinal colonization of infants.

Objectives: This research aimed to develop Sangyod rice protein-based infant formula for cow milk allergic infants, evaluated its nutritional composition, sensory, and anti-allergenic activity, and investigated the effects of the formula on gut microbiota modulation.

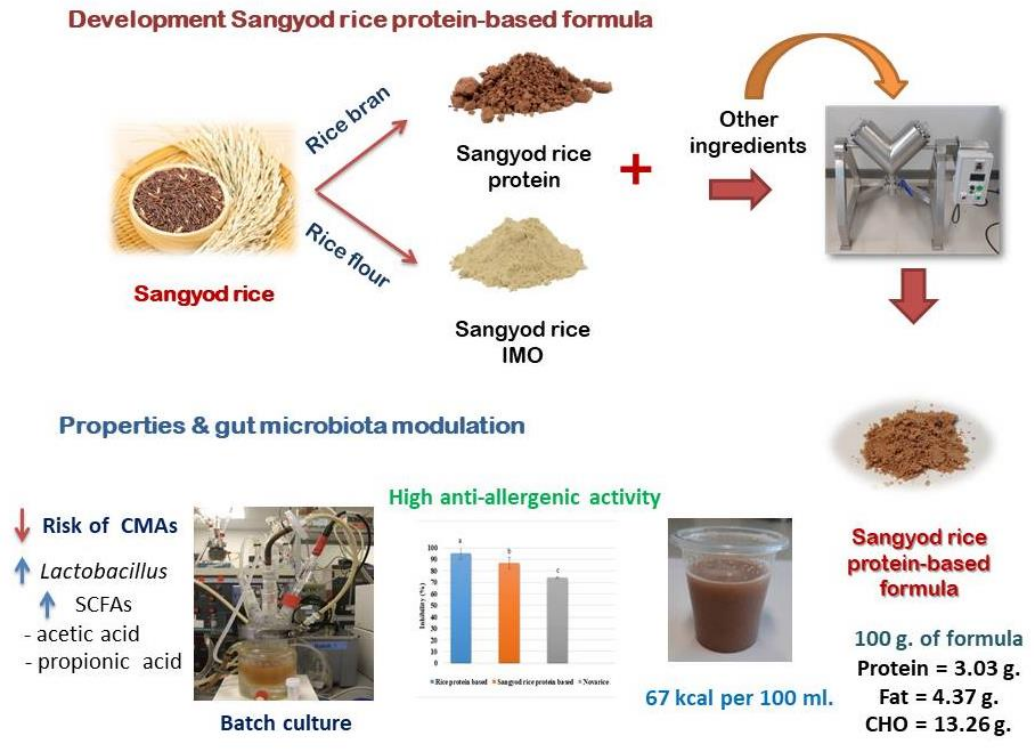
Materials and methods: The development of infant formula based on Sangyod rice protein and fortified with IMO from Sangyod rice flour was studied on nutrition composition, microbiology, anti-allergenic activity, and effect on gut microbiota. These properties reflect quality and safety to meet requirements of infant and follow-up formula.

Results: The energy of Sangyod rice-protein-based formula was 67 kcal per 100 ml. The results indicated that the formula met the requirements of macronutrients providing 3.03 g of protein, 4.37 g of fat, and 13.26 g of carbohydrate, and

passed on total bacteria contamination. Surprisingly, the developed formulas showed higher results in an anti-allergenic activity test (86.98±5.49%) by inhibition on the release of β-hexosaminidase enzyme in RBL-2H3 cells compared to a commercial hypoallergenic formula. The addition of prebiotic (IMO) significantly increased populations of *Lactobacillus* (10.7 log cell/ml) within 24 hours ($p<0.05$) compared with commercial product. This result affected the production of shot chain fatty acids (SCFAs) by increasing the amount of acetic acid and propionic acid, but had no effect on lactic acid and butyric acid production. This result may have a beneficial effect on the immune system and hopefully can help to prevent or decrease risk of cow milk allergy. The sensory evaluation of Sangyod rice protein-based formula showed the highest scores in test (6.34±1.39), odor (6.74±1.15) and overall acceptability (6.52±1.26), but had no significant differences ($p<0.05$) compared to commercial hypoallergenic formula.

Conclusion: The Sangyod rice protein-based formula can compete and was acceptable for the hypoallergenic formula and would be an option for substituting cow milk in the treatment of cow milk protein allergy. However, this study is the first step to develop the hypoallergenic formula and still needs the preclinical testing and clinical study in the future to claim as a hypoallergenic formula.

Keywords: Sangyod rice, Infant formula, rice protein-based formula, hypoallergenic formula



INTRODUCTION

Food allergy is an adverse reaction of the immune system, the prevalence of which is greater in young children than adults, especially during the first year of life because of immune system and gastrointestinal tract immaturity [1]. Cow milk and eggs are the most common causes of food allergies. In Thailand, cow milk allergy has been the leading cause of food allergy in infants and

young children for a long time, and the severity is increasing continuously. The prevalence of food allergies in Thai children is 6.25% and approximately 0.45%for the IgE-mediated food allergies [2]. The World Health Organization (WHO) recommends that all infants should be attempted to exclusively breastfeed for the first 6 months of life, but the substitution with formula is needed when breast milk is insufficient or lacking. An alternative hypoallergenic milk substitute is necessary during infancy and early childhood when cow milk formula is intolerant [3]. Hypoallergenic formulas are the ones that are clinically tolerable in infants. The available allergen-reduced dietary products for infants are derived from different sources of protein, such as casein, whey, soy, and rice exposed to enzymatic hydrolysis and further processing. The hydrolyzed formula may decrease risk of food allergy by changes qualitative of the peptide to the epitope. The exposure of the smaller peptide to gut-associated lymphoid tissue (GALT) is thought to induce oral tolerance without sensitization, as the decreased molecular weight has been associated with the decreased allergenicity of the protein [4].

Rice is the one of the less allergenic staple foods, the reaction of which is lower than 1 % of allergic children [5]. Rice protein is the one of hypoallergenic food ingredients used as an alternative plant-based protein in nutritional products [6], although the rice protein-based formulae are limited in the market. In addition, the rice variety is not the same in this study. Sangyod rice (*Oryza sativa*, L., var. *indica*) is native to southern Thailand and was originally planted in Phatthalung province for hundreds of years. It has been reported to have a high content of minerals, vitamin B complex, and bioactive compounds that can protect against oxidative stress related chronic diseases such as diabetes mellitus, cardiovascular disease, and cancer [7]. A major concern about rice-based products is the arsenic content in rice. In this study, the Sangyod rice was obtained from Phatthalung province, and the subsequent study of heavy metal in six districts of the province showed that inorganic arsenic levels were lower than the maximum limit and safe for consumption [8]. However, the testing of arsenic content is needed in the rice and/or finished formula.

Isomaltooligosaccharides (IMO) are accepted as prebiotics and are applicable as food ingredients because of their ability to enhance the physiochemical quality of foods as anti-fading agents for food colors, as food antioxidants, as sweeteners, and as functional ingredients [9]. Additionally, these oligosaccharides have multiple physiological functions, including the enhancement of gut microflora based on the selective proliferation of bifidobacteria stimulation [10-11]. They are also correlated with a lower risk of infections and diarrhea, and an amelioration of the immune system reaction [12]. The Food and Agricultural Organization (FAO) of the United Nations supports the supplementation of formula with prebiotics in infants aged five months and older, as these infants will have a mature immune system and intestinal colonization [13]. For these reasons, an alternative Sangyod rice protein-based formula fortified with IMO form Sangyod rice flour would be an option for substituting cow milk in the treatment of cow milk protein allergy.

MATERIALS AND METHODS

Sangyod rice protein sample preparation: Sangyod rice bran was extracted in alkaline conditions at pH 8.0 at 50 °C for 5 min, followed by ultrasonic extraction at the power level P = 345 W for 5 min and autoclave at 130 °C for 60 min. For rice bran protein, hydrolysate from sangyod rice was derived from digestion with alcalase enzyme for 2 hours. The protein hydrolysated encapsulation by spray drying with maltodextrin 1:10 w/w at 185 °C [14-15].

Sangyod rice isomaltooligosaccharide (IMO) preparation: Sangyod rice slurry was prepared at a concentration of 25% (w/v) with addition of 0.05% α-amylase and was continuously agitated at 100 rpm at a

pH 6 at 90˚C for 30 minutes. Afterwards, 0.08% β-amylase and 0.1% transglucosidase-L was added (pH 5.5), and the slurry was held at 60˚C for 48 hours and powdered by spray dryer.

The other ingredients in the formula, such as maltodextrin, were purchased from Chemipan Corporation Co., Ltd. (Bangkok, Thailand). The premixed vitamins and minerals and medium chain triglyceride (MCT) oils were purchased from Adinop Co., Ltd. (Bangkok, Thailand). The amino acids, fish oil, taurine, and sunflower lecithin were purchased from an online store (www.108vitamin.com). The rice bran oil, canola oil, and soybean oil (as source of vegetable oil) were obtained from Tesco Lotus, Hatyai, Songkhla, Thailand. All ingredients used for formulation in the study were food grade additives to ensure on safety for human consumption.

Sangyod rice protein-based formula preparation: The development of Sangyod rice protein-based formula consisted of studying the regulation of infant follow-on formula [16-17] and estimating the macronutrient concentrations and other ingredients such as vitamins and minerals. To reach the regulation, all the ingredients were weighed and mixed by a V-shape mixer. The composition of ingredients in 100 grams of the formula was prepared as follows: 45 g Maltodextrin; 15 g Sangyod rice protein; 11.5 g Soy bean oil; 10 g Sangyod rice IMO; 5 g premixed vitamins and minerals; 4 g MCT; 2.5 g Canola oil; 2.5 g Sunflower lecithin; 2 g Rice bran oil; 1 g Amino acid complex; 0.5 g Guagum; 0.6 mg L-Lysine; 0.12 mg L-Threonine; 0.1 g Fish oil; 0.1 mg Choline & Inositol; 0.07 g Sodium chloride; 0.045 mg Vitamin A; 0.04 mg Taurine; 0.04 mg L-carnitine; 0.03 mg Vitamin D3; and 0.01 mg Vitamin K. The minor ingredients (vitamins, minerals, etc.) were mixed, followed by adding Sangyod rice protein and carbohydrate sources (maltodextrin and Sangyod rice IMO). After that, oil soluble vitamins, MCT oil, vegetable oils, and emulsifier were incorporated via dry blending process.

The nutrition composition analysis: The nutritional composition of the formula was analyzed by the Central Laboratory (Thailand) Co., Ltd. (Songkhla, Thailand). The methods used for analysis of energy, macronutrients, and micronutrients were performed following international standards as shown in Table 1.

Table 1: Nutrients and reference methods used for analysis of Sangyod rice protein-based formula

Nutrient	Reference method
Energy, Carbohydrate	Journal of AOAC International (1993)
Protein	In-house method TE-CH-042 based on AOAC (2019) 981.10
Fat	AOAC (2019) 948.15
Vitamin A	In-house method based on Chemical and Technical Assessment (2004)
Vitamin D	In-house method based on Journal of Chromatography A. 1105 (2006) 135-139
Vitamin E(α-tocopherol)	Liquid Chromatographic Analysis of Food and Beverage. Vol2. 1997
Vitamin K1	Based on AOAC (2016) 999.15
Vitamin B1, Vitamin B2, Niacin, Vitamin B6, Folic acid, Pantotinic acid, Vitamin B12, Biotin, Vitamin C	In-house method based on Analytica Chimica Acta 569 (2006) 169-175
Iodine	Based on AOAC (2016) 992.22
Chloride	In-house method STM No.03-096 based on AOAC (2016) 937.09
Calcium, Phosphorus, Magnesium, Iron	In-house method TE-CH-134 based on AOAC (2019) 984.27
Sodium, Potassium, Zinc	In-house method TE-CH-134 based on AOAC (2019) 984.27

The microbial contamination: The microbial test of the formula was determined by the Central Laboratory (Thailand) Co., Ltd. (Songkhla, Thailand). Total viable plate count and yeasts and molds were performed by pour plate method. FDA BAM (2001) and *E. coli* was performed via MPN method series 3:3:3. FDA BAM (2017) was in accordance with the standard of the Notification of the ministry of public health of Thailand (No.171) B.E. 2539 (1996) infant food and food of follow up formula for infant and young children (No.2).

Anti-allergenic activity: The anti-allergenic activity of the formula was tested by inhibitory effects on the release of β-hexosaminidase in rat basophilic leukemia RBL-2H3 cells (RBL-2H3 cells). The method used was a modified method of Matsuda [18].

Sensory evaluation: Sensory evaluation was carried out by 50 semi-trained panelists. The Sangyod rice protein-based formula was evaluated in appearance, homogeneity, odor, taste, and overall acceptability with a 9-point Hedonic scale. The score of each parameter was compared with a commercial hypoallergenic formula (Novarice®) and developed rice protein-based formula from commercial rice protein. The sensory evaluation was performed in a standard sensory laboratory equipped with individual cabinet. The test sample preparation used 4.5 g of the formula with 30 ml of warm water (40ºC). The test sample was served with white bread and fresh water.

Effect of Sangyod Rice protein-based formula on gut microbiota: *Step 1: Upper gut digestion before in vitro fermentation:* samples were enzymatically digested mimicking the upper gut (mouth, stomach, and the small intestine). The conditions of digestion were mimicked according to the method of Jensen [19]. The retentate (non-digestible sample) was lyophilized by a freeze dryer and stored at -20˚C until use for batch fermentation study. *Step 2: In vitro fecal batch fermentation*: fresh feces was collected from four healthy volunteers who usually ingested a normal diet, presented no digestive tract disease, and had not received antibiotics, prebiotics, or probiotics for at least 3 months. Fresh feces were immediately put in an anaerobic chamber and homogenized with 10% (w/v) of the fecal material in 0.1 M phosphate-buffered saline (PBS) at a pH 7.0. The slurry was filtered through a 1-mm sieve using a homogenized bag, and the fecal slurry was immediately used as inoculum. Batch fermentation was carried out in water-jacket glass vessels at 37˚C, which were filled with 90 ml of pre-sterilized basal culture medium and inoculated with 10% (v/v) of fecal slurry [20]. The freeze-dried pre-digested samples were aseptically added to each vessel to give a final concentration of 1% (w/v). The last vessel served as control (basal medium and fecal slurry without sample). The temperature of the vessel was controlled at 37ºC using a circulating water bath. The sample in each vessel was magnetically stirred with a magnetic bar and maintained a pH at 6.8±0.2 via a pH controller. Nitrogen gas was sparged constantly at 15 ml min^{-1} through the vessel to maintain anaerobic condition. Samples were

taken from each vessel at 0, 12, 24, and 48 hours of fermentation for enumeration of fecal bacteria and analyses of short chain fatty acids (SCFAs), as well as lactic acid by enumeration of fecal microbiota by fluorescent *in situ* hybridization **(**FISH) and high-performance liquid chromatography **(**HPLC), respectively.

Statistical analysis: A t-test was used for statistical analysis of the samples. Analysis of variance (ANOVA) and mean comparisons were performed using the Duncan's new multiple range test (DMRT). Statistical analyses were carried out using the SPSS statistical software (SPSS, Inc., Chicago. IL version 21).

RESULTS AND DISCUSSION

Nutrition composition: Nutrition composition of Sangyod rice protein-based formula was analyzed by standard methods to ensure that the developed formula met the requirements of CODEX and Thai FDA for infant and follow-up formula. The energy of the formula was 446 kcal per 100 g of formula powder or 67 kcal per 100 ml. Normally, the commercial hydrolysate rice protein formula in the market was 68-71 kcal [21]. The formula was in accordance to the standard energy density of follow-up formula and formulas for special medical purposes intended for infants (CODEX STAN 72 – 1981) amended in 2015 [22]. It was also in accordance with standards for follow-up formula (CXS 156-1987) amended in 2017 and the requirement of Notification of the Ministry of Public Health of Thailand (No.171) B.E.2539 (1996): infant food and food of follow-up formula for infant and young children (No.2). The recommended energy shall provide 60-85 kcal per 100 ml. The results indicated that the formula met the requirements of macronutrients providing 3.03 g of protein, 4.37 g of fat, and 13.26 g of carbohydrate as shown in Table 2. The micronutrients were mostly appropriated for substitution of cow's milk. However, the amount of vitamin B (niacin, folic acid, and pantothenic acid), fat soluble vitamin A and vitamin D, and minerals in premixed vitamin used in the formulation did not provide the appropriate amount to meet the requirement of standard follow-up formula. The nutritional inadequacy and insufficiency were the major concerns of using rice-based formula for cow milk substitution because of extremely low protein content in rice milk [23]. However, the nutritional value in a rice-based formula of several studies have shown normal growth patterns in cow milk allergic infants [24] and most of the studies found no important deficits in growth parameters in children who adopted rice-based formula compared to soy formula and cow milk formula [25-27]. The study on healthy infants fed with rice protein-based formula until complementary feeding showed normal growth, weight, and similar plasma concentrations of calcium, magnesium, and alkaline phosphatase compared to standard formula [28]. However, there is lacking evidence of long-term use in infants, especially for bone mineralization. Hence, long-term clinical studies may need to be investigated to confirm this health effect in infants and children.

Table 2: Nutrition composition in rice protein-based formula

Nutrient	Nutrient/100 g	Nutrient /100 kcal	Requirement /100 kcal
Protein	13.53 g	3.03 g	3.0 – 5.5 g
Fat	20.41 g	4.37 g	3.0 – 6.0 g
Carbohydrate	59.29 g	13.26 g	9.0 –14.0 g
Vitamin A (Retinol)	Not detected	Not detected	75 – 225 µg
Vitamin D	Not detected	Not detected	40 – 120 IU
Vitamin E (α-tocopherol)	9.14 IU	2.05 IU	≥ 0.70 IU
Vitamin K1	1330 µg	298 µg	≥ 4 µg
Vitamin B1	1.18 mg	264.40 µg	≥ 40 µg
Vitamin B2	1.56 mg	349.55 µg	≥ 60 µg
Niacin	0.30 mg	67.22 µg	≥ 250 µg
Vitamin B6	0.32 mg	71.70 µg	≥ 45 µg and ≥ 15 µg /g protein
Folic acid	0.01 mg	2.24 µg	≥ 4 µg
Pantothenic acid	0.33 mg	73.94 µg	≥ 300 µg
Vitamin B12	0.00428 mg	0.96 µg	≥ 0.15 µg
Phosphorus	874.51 mg	195.95 mg (Ca:P = 1.4:1)	≥ 60 mg (Ca:P = 1-2:1)
Magnesium	68.77 mg	15.40 mg	≥ 6 mg
Iron	35.32 mg	7.91 mg	1-2 mg
Iodine	286 µg	64.08 µg	≥ 5 µg
Zinc	17.31 mg	3.87 mg	≥ 0.15 mg
Biotin	1.81 mg	391.73 µg	≥ 0.15 µg
Vitamin C	0.153 mg	0.34 mg	≥ 8 mg
Sodium	168.57 mg	37.77 mg	20 – 85 mg
Potassium	255.81 mg	57.32 mg	≥ 80 mg
Chloride	370 mg	82.90 mg	≥ 55 mg
Calcium	1258.23 mg	281.93 mg	≥ 90 mg
Energy	446.28 kcal = 66.94 kcal/100 ml		60-85 kcal/100 ml

Microbial determination: The microbiological quality is one of the most important parameters for infant formula production to evaluate the safety of different infant formula products [29]. The microbial quality of the formula was determined according to the Notification of the Ministry of Public Health of Thailand (No.171) B.E.2539 (1996): infant food and food of follow-up formula for infant and young children (No.2), [17]. Infant formula contains rich sources of nutrients to support bacterial growth and has the potential risk of foodborne pathogens. Infants are susceptible to infection by such pathogens because of incomplete immune system development and a lack of competing intestinal flora [30]. Total viable plate count of the formula was < 250 CFU/g. The number of yeasts and molds were less than 10 CFU/g. The number of *E. coli* was less than 3.0 MPN/g, meaning the report did not find colonies of yeasts and molds on the culture plate and *E. coli* was not detected. These results indicated that the rice protein-based formula passed in regard to total bacteria contamination.

Anti-allergenic activity test: The rice protein component is generally regarded as hypoallergenic [31]. Rice protein hydrolysates have been used in several European countries for special medical purposes such as treatment of cow milk protein allergy since early 2000s [21]. An anti-allergenic activity test of 3 rice protein-based formulas (Sangyod rice protein-based formula, commercial hypoallergenic formula (Novarice®), and developed rice protein-based formula from commercial rice protein) showed that all formulas exhibited anti-allergenic activities via inhibition of the release of β-hexosaminidase enzyme from rat basophilic leukemia cells (RBL-2H3 cells). The percentage of inhibition was highest in the developed rice protein-based formula (95.43±5.83%), Sangyod rice protein-based formula (86.98±5.49%), and a commercial Novarice® (74.06±0.95%), respectively. Therefore, all three formulas tested had anti-allergenic activity and can compare with the commercial hypoallergenic formula (Novarice®) for infants and young children who have had allergies to cow milk protein. However, more preclinical testing and clinical studies are needed in the future to claim these formulas as hypoallergenic formulas.

Sensory Evaluation: Infants with cow's milk allergy mostly have feeding difficulties due to eliminating aspects of their diet compared to children consuming an unrestricted diet [32]. The study of overall acceptability in rice protein-based formula showed promising results [27], but a study by Vandenplas reported 18.8% of parents felt that their infant dropping out of the formula [33] could have been due to the bitter test and foul-smell of hydrolysated rice protein [34]. Sensory evaluation of Sangyod rice protein-based formula and developed rice protein-based formula from commercial rice protein compared with commercial hypoallergenic formula (Novarice®) is shown in Table 3. The Sangyod rice protein-based formula showed the highest scores in testing (6.34±1.39), odor (6.74±1.15), and overall acceptability (6.52±1.26), but showed no significant differences ($p<0.05$) from commercial hypoallergenic formula (Novarice®). However, the scores in appearance and homogeneity were significant differences lower than the commercial formula, and the scores of developed rice protein-based formula was the lowest in all parameters tested. Thus, the Sangyod rice protein-based formula can compete and was acceptable for the hypoallergenic formula. The most important ingredient in the formula was the source of protein because when comparing tested parameters between Sangyod rice protein-based formula and developed rice-protein based-formula the source of protein was the sole significant difference recorded.

Table 3: Sensory evaluation of rice protein-based formula

Properties	Sangyod rice protein-based formula	Rice protein-based formula	commercial hypoallergenic formula
Appearance	6.54±1.11 [b]	6.04±1.63 [c]	7.84±.95 [a]
Homogeneity	6.26±1.35 [b]	5.94±1.62 [b]	7.96±.86 [a]
Odor	6.74±1.15 [a]	5.54±1.78 [b]	6.66±1.54 [a]
Taste	6.34±1.39 [a]	5.29±1.76 [b]	5.90±1.78 [a, b]
Overall acceptability	6.52±1.26 [a]	5.48±1.56 [b]	6.52±1.48 [a]

Effect of Sangyod rice protein-based formula on gut microbiota: The effect of Sangyod Rice protein-based formula and commercial product (Novarice) on gut microbiota evaluated by fecal batch fermentation is shown in figure 1. The levels of different bacterial groups by FISH were decreased in populations of *Bifidobacterium* and significantly increased populations of *Lactobacillus* (10.7 log cell/ml) within 24 hours ($p<0.05$) compared with commercial product (Novarice®). However, the number of all unbeneficial bacteria (*Bacteroides*, *Eubacterium,* and *Clostridium*) increased as well, and this result affected the production of short chain fatty acid (SCFAs), shown in table 3, because IMOs have been shown to promote the growth of *Lactobacillus* and *Bifidobacterium*. Several studies evaluated the effect of prebiotic-supplemented formula on the intestinal microbiota in infants, showing the stimulating effect on the intestine by selectively supporting the growth of *Bifidobacterium* and *Lactobacillus* strains, similar to breast milk feeding [35]. This study shows that the rice protein-based formula is safe and supports beneficial gut microbiota due to its metabolite short-chain fatty acids (SCFA) [36]. The number of acetic acids and propionic acids were significantly increased in all groups, but the number of lactic acids and butyric acids could not be detected after 24 hours. However, the number of short chain fatty acids was highest in the Sangyod rice protein-based formula group. SCFAs have been shown to exert many positive effects on mammalian energy metabolism. The important roles of SCFAs are acting as an energy source of epithelial cells, acting as modulators of colonic and intracellular pH, as well as gene expression and associations with the nervous system linked to the gut-brain axis [37]. Propionate and acetate also play key roles in the regulation of the expression of immune system genes. Studies have shown that lymphocytes harvested from LNs and cultured with acetate, propionate, and butyrate produced high levels of IL-10, whereas butyrate alone had little effect [38]. For this reason, the addition of IMO in the formula may have a beneficial effect on the immune system. The Sangyod rice protein-based formula developed is a functional food product (FFP) according to the definition and process of development described by Martirosyan *et al* [39]. However, evidence regarding the use of prebiotics in the prevention of allergies is inconclusive and still requires more future study.

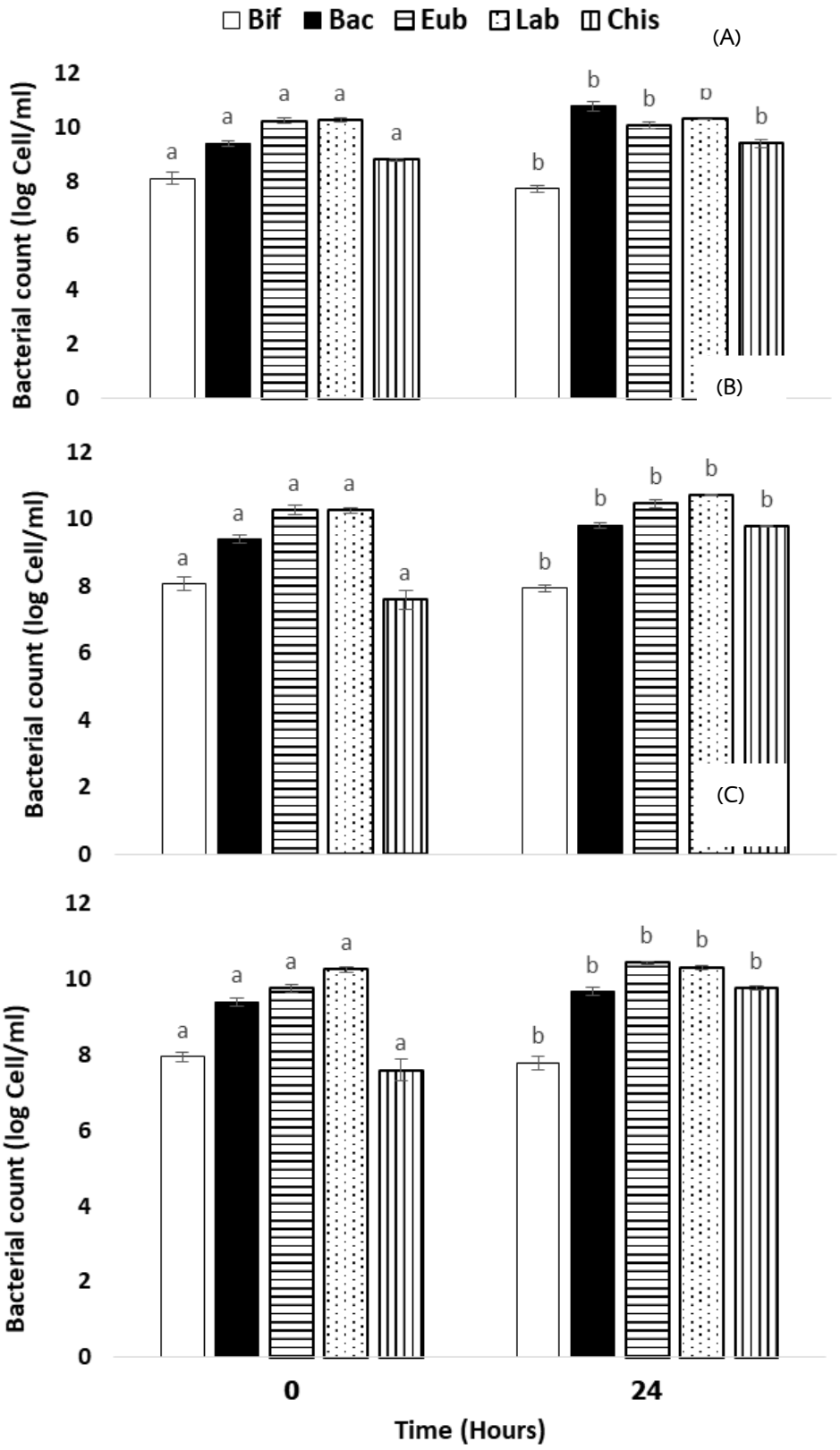


Figure 1. Bacterial populations (log cell ml^{-1}) in fecal batch culture at 0 and 24 hr. (A = control, B= Sangyod rice protein-based formula, C= commercial product)

Table 4: Acetic acid, lactic acid, propionic acid and butyric acid production in fecal batch culture

Time	Acetic acid (mM)			Lactic acid (mM)			Propionic acid (mM)			Butyric acid (mM)		
	C	CPN	SRP	C	CPN	SRP	C	CPN	SRP	C	CPN	SRP
0	-	-	-	-	-	-	-	-	-	-	-	-
6	10.6±0.3[a]	52.6±0.3[a]	54.4±0.5[a]	-	-	-	-	33.2±0.1[a]	35.6±1.5[a]	-	-	-
12	18.6±0.0[b]	71.8±0.3[b]	83.3±9.4[c]	-	4.3±0.01[b]	5.9±0.2	-	47.1±0.3[b]	49.8±1.4[b]	-	-	-
24	26.7±0.2[c]	80.7±0.8[c]	83.7±1.0[b]	-	2.9±0.01[a]	-	8.6±0.1[a]	51.8±0.4[c]	62.6±2.7[c]	-	7.73±0.01	-
48	42.2±0.0[d]	95.9±0.4[d]	106.4±5.4[d]	-	-	-	11.6±0.0[b]	61.0±0.0[d]	68.6±0.4[d]	-	-	-

Notes: C=Control, CPN= Commercial product-Novarice, SRP=Sangyod Rice Protein-based formula

CONCLUSION

The development of infant formula based on Sangyod rice protein and fortified with IMO from Sangyod rice flour showed safety and nutrititional value confirmed by nutrition composition and microbial determination. Surprisingly, the developed formulas were higher in anti-allergenic activity than a commercial hypoallergenic formula and the addition of prebiotic (IMO) increased the beneficial bacteria that affects the production of shot chain fatty acids**,** which may have a beneficial effect on the immune system and hopefully can help to prevent or decrease risk of cow milk allergy. However, this study is the first step to develop the hypoallergenic formula from Sangyod rice protein and further animal and clinical studies are needed to be claimed in the future as a hypoallergenic formula.

Abbreviations: CFU: Colony forming unit, *E. coli:* Escherichia coli, FISH: Fluorescent *in situ* hybridization, HPLC: High Performance Liquid Chromatography, IgE: Immunoglobulin E, IMO: Isomaltooligosaccharide, MCT: Medium Chain Triglyceride, MPN: Most probable number, RBL-2H3 cells: Rat Basophilic Leukemia-2H3 cells, SCFA: Shot Chain Fatty Acid

Author contributions: M. Madtohsoh and P. Detarun designed and conduct the experiments. S. Wichienchot provided supervision throughout this work. M. Madtohsoh wrote the manuscript. S. Wichienchot and P. Detarun critically revised the manuscript and approved the final version. All authors contributed to the article and approved the submitted version.

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