DEVELOPMENT OF HIGH-PROTEIN PLANT-BASED YOGURTFROM OAT, CHICKPEA AND PEA PROTEIN

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Abstract.

For health benefit and sustainable process reasons, consumption of plant-based diets is becoming more popular nowadays. Yogurt, traditionally fermented from dairy milk, hasalready been developed with plant milks offering a dairy-free alternative for individuals with lactose intolerance and cow's milk allergy. In this study, oat and chickpea were selected to create a new plant-based yogurt. A more balanced amino acid profile can be achieved by combining a grain (oat) and a legume (chickpea) in the product. The first objective was to investigate the appropriate proportion of oat and chickpea (5:5, 6:6, 7:7) in yogurt. Sensory analysis revealed that the 6:6 ratio was favored by panelists in more quality attributes ($p \leq 0.05$). Most plant-based foods are low in protein compared with animal-derived foods. Hence, the second objective was to enhance the protein concentration of yogurt by substituting some chickpea with pea protein isolate (4:2, 3:3, 2:4). The higher level of pea protein in yogurt contributed to decreased firmness, lower acidity, and higher syneresis($p \leq 0.05$). The protein contents of yogurt substituted with 0, 2, 3 and 4% pea protein were 3.74, 5.58, 6.71 and 7.98%, respectively. However, yogurt with 3% pea protein was preferred in more sensory quality categories ($p \leq 0.05$). In summary, the combination of 6% oat, 3% chickpea, and 3% pea protein was the most suitable ingredient ratio for developing a novel plant-based yogurt with a high-protein content and a balanced amino acid composition.

Keywords: High-protein, Plant-based yogurt, Oat, Chickpea, Pea protein

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Introduction

Yogurt, also spelled yoghurt or yoghourt, is one of the most ancient foods in history. It was discovered accidentally when milk became in contact with intestinal components, it later turned sour and partially coagulated. Dating back to 6,000 B.C., the health benefits of fermented milk consumption were already written in old Indian documents (Fisberg & Machado, 2015). The traditional yogurt starter cultures, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, are the main reasons for health-promoting effectsof yogurt consumption. They produce bacteriocins which exhibit antimicrobial activity against pathogenic bacteria including *Salmonella*, *Shigella*, *Escherichia coli*, and *Pseudomonas* (Dave & Shah, 1997). The two yogurt organisms are also considered to act as probiotics. They have been scientifically demonstrated to resist the human gastrointestinal conditions and remain sufficiently viable to exert health benefits such as decreasing the occurrence of diarrhea, stimulating the host's immune system, etc. (Chandan *et al*., 2017).

Among yogurt variations, plant-based yogurt is a fast-growing product category with a projected growth rate of nearly 20% from 2020 to 2027 (Grand View Research, 2019). As animal-derived foods raise some concerns regarding unhealthy nutritional composition, environmentally unfriendly production process, and animal ethics, more consumers gradually adopt plant-based lifestyles leading to the rising demand for plant-based food choices (Boeck *et al*., 2021). In comparison with dairy-based yogurt, plant-based yogurt alternatives, made with pulses and cereals, contain lower saturated fats, higher dietary fibers, and functional phytochemicals (Craig & Brothers, 2021). Furthermore, non-dairy yogurt can be a great option for individuals with lactose intolerance and cow's milk protein allergy. From a nutritional point of view, diets from plant ingredients still have a major disadvantage relatedto low protein content resulting in incomplete amino acid profile compared with food from animal sources (Moyer & Mitchell, 2021). In terms of micronutrients, consumers exclusively eating plant-derived foods over an extended period of time may be deficient in calcium, vitamin D, and vitamin B12 (Melina *et al*., 2016).

In order to develop a new plant-based food, production strategies to correct the nutritional shortfall should be applied. For plant-based yogurt, only soy, almonds, and coconut have beenused as primary raw materials in Thailand. In the United States, more varieties of yogurt have already come out in the market based upon coconut, almonds, oats, cashews, soy, pea, hemp, pumpkin seeds, fava beans, pili nuts, and the blend of these bases (Craig & Brothers, 2021).

To diversify plant-based yogurt options, chickpea is another nutritious pulse which can be employed as the principal ingredient in yogurt. Benefits of chickpea intake include long-term satiety, postprandial blood glucose control, enhanced insulin sensitivity, and inhibition of cholesterol absorption (Aisa *et al*., 2019). However, it is rather difficult to formulate yogurt toachieve the same or higher protein quality compared to dairy yogurt with chickpea alone. Legumes including chickpea are naturally low in methionine and cysteine. To improve the product's protein quality, chickpea should be paired with grains, such as oats and rice, which are more abundant in methionine. On a similar note, grains, limited in lysine, can be complemented with chickpea which is a good source of lysine to obtain the product with a more balanced amino acid composition (Bonke *et al*., 2020; Landi *et al*., 2021).

In relation to protein quantity, the majority of plant-based yogurt currently available in the market contains 3-5 g protein/serving whereas dairy-based version generally contains higher protein (more than 5 g/serving) (Craig & Brothers, 2021; Grasso *et al*., 2020). To boost the protein level in plant-based yogurt, plant protein isolates, such as pea and soy protein, can be incorporated into the recipe. Pea protein can be an interesting component to create a high- protein yogurt offering the soy-free alternative for people with soy allergy. Moreover, a combination of pea and oat in the same product provided the advantages of improved amino acid concentrations and enhanced sensory attributes (Bonke *et al*., 2020). According to the regulation by the Ministry of Public Health of Thailand, the minimum protein content of 10 g/serving is required in order to declare a nutrient claim of high protein on the productlabel (Notification of the Ministry of Public Health, 1998).

In the present study, a novel blend of plant raw materials was exploited to develop a high- protein plant-based yogurt consisting of oat, chickpea, and pea protein isolate. The first aim was to investigate the appropriate proportion of oat and chickpea in the yogurt. The second part of the study was aimed at increasing the protein content of the developed yogurt. The proper level of chickpea substitution with pea protein isolate was evaluated. The final plant-based yogurt recipe was chosen considering sensory and product quality data.

Methods

Materials

Whole grain oat flour (McGarrett), natural cane sugar (Mitr Phol brand), and pure vanilla extract (McCormick) were purchased locally in Tops supermarket, Thailand. Chickpea flour (Organica brand) and carrageenan (supplied by Krungthep Chemi) were bought from Shopee online marketplace. Pea protein isolate (90%) was provided by Qualite Ingredients. Freeze- dried starter cultures (SYAB1 from Sacco System) comprising *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *Bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* spp. *Lactis* were bought directly from Tinnakorn Chemical and Supply. Peptone and *Lactobacillus* De Man, Rogosa and Sharpe (MRS) agar were acquired from HiMedia.

Production of plant-based yogurt

Oat flour and chickpea flour were formulated into three recipes (5:5, 6:6, 7:7). Each flour type was separately dissolved in water. In a different bowl, cane sugar (7%) was dissolved, then mixed with vanilla extract (2.5%). The components were put together in a kitchen blender for 1 min. In a small bowl, carrageenan (0.1%) was solubilized with warm water. It was then added to the rest of the ingredients prior to homogenization (T 25 digital Ultra- Turrax®, IKA brand, Germany) at 8,000 rpm for 5 min. The mixture was subjected to pasteurization at 72°C for 1 min followed by cooling in an ice bath. Once the yogurt mix chilled to about 40°C, the starter cultures were sprinkled evenly onto the mix. The fermentation was carried out at 42°C for 6 h. The resulting yogurt was kept in a refrigerator overnight to stabilize its texture before further analysis.

For the subsequent section of the study, the proportion of oat and chickpea (6:6) was selected as the recipe base due to its highest acceptance in sensory evaluation. To develop a high-protein recipe, chickpea was partially substituted with pea protein in three differentratios (4:2, 3:3, 2:4). The rest of the production procedures was the same as before.

Physical analysis of yogurt

The firmness of yogurt (N) was assessed using Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) by the modified procedures from Mousavi et al. (2019). Yogurt was placed in a glass sample cup. The sample height was at least 4 cm. The cylindrical probe was used to penetrate the sample to the depth of 2 cm. The probe speed range was 2 mm/s.

The color values of yogurt (L*, a*, b*) were measured using Hunter Lab spectrophotometer (ColorQuest® XE, Hunter Associates Laboratory, USA) by the method modified from Achayuthakan *et al*. (2018). Yogurt was put in a clear plastic bag and flattenedinto a sheet. The product color was evaluated in RSEX mode using CIELAB system.

Chemical analysis of yogurt

The pH of yogurt was measured using a pH meter (Starter 3100 pH Bench, Ohaus, China) by the method as described in Igbabul *et al*. (2014). 10 g of yogurt was mixed with 100 ml of distilled water, and stirred until a homogeneous mixture was obtained. The sample pH was determined using recently calibrated pH meter.

The titratable acidity of yogurt was assessed following the procedures by Oladipo *et al*. (2014). 10 g of yogurt was blended with 30 ml of distilled water in a flask, and shaken to ensure homogeneity. The sample flask was then titrated against 0.1 N NaOH standard solution using phenolphthalein as an indicator. The titratable acidity (%) was calculated basedon lactic acid concentration in yogurt using the following equation.Titratable acidity (%) = [volume of NaOH (ml) x NaOH concentration (N) x 0.09 x 100] / sample weight (g)

The syneresis of yogurt was evaluated using the same method as Tan & Korel (2007). 10 g of yogurt was centrifuged (Universal 320 Benchtop Centrifuge, Hettich Instruments, Germany) at 5,000 rpm for 20 min. The supernatant liquid was taken out and weighed. The degree of syneresis (%) was calculated using the below equation. Syneresis (%) = [liquid fraction weight (g) x 100] / sample weight (g)

The protein content of yogurt was measured using the Kjeldahl method (AOAC, 2000). 1 gof yogurt was digested with 25 ml of concentrated H_2SO_4 in the presence of 10 g of K_2SO_4 and 1 g of $CuSO_4$. 200 ml of distilled water was put in the cooled digestion flask. It was then placed in a distillation unit together with a flask of 4% boric acid (H_3BO_3) added with a mixedindicator of bromocresol green and methyl red. The flask with boric acid was titrated against 0.1 N HCl solution. The protein content (%) was calculated using the below equation. Protein (%) = [(volume of HClsample-volume of Hclblank) (ml) x HCl concentration (N) x 1.4 x 6.25] / sample weight (g)

Microbiological analysis of yogurt

Lactic acid bacteria (LAB) count was determined following the procedures by AOAC (1998). 25 g of yogurt and 225 ml of 0.1% peptone were transferred to a stomacher bag, and homogenized using a stomacher for 1 min. The homogenized sample was serially diluted to 10^{-3}, 10^{-4} and 10^{-5} dilutions. Each dilution (0.1 ml) was then spread onto MRS agar plate. The plates were incubated anaerobically at 37°C for 24-48 h. Colonies were enumerated and expressed in log Colony Forming Unit (CFU)/g.

Sensory analysis of yogurt

Organoleptic properties of yogurt were assessed in aspects of appearance, color, aroma, sweetness, sourness, viscosity, mouthfeel, and overall liking. The affective test was conductedfor yogurt evaluation using a 9-point hedonic scale (scores of 1=dislike extremely and 9=like extremely). The panelists were untrained 30 people with one criterion of consuming any kindsof yogurt at least a few times per month.

Statistical analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation. Completely Randomized Design (CRD) was applied to all analyses except sensory evaluation using Randomized Complete Block Design (RCBD). Duncan's New MultipleRange Test (DMRT) was employed to compare the means using IBM SPSS 26.0 software.

Results and Discussion

Effect of the proportion of oat and chickpea on the quality and acceptance of yogurt

To devise a new plant-based yogurt with a more balanced amino acid composition, oat and chickpea were selected as grain and pulse representatives for complementing individualamino acids. The suitable proportion of the main two ingredients in yogurt was investigated.

Three recipes were attempted with oat and chickpea at the concentrations of 5:5, 6:6 and 7:7 (%:% w/w). According to texture analysis, the firmness or hardness of yogurt escalated as the combined amount of oat and chickpea increased (Table 1). The recipe with 7:7 ratio displayedthe exceptionally high firmness of 4.93 N compared with the rest of the samples ($p \leq 0.05$).

Table 1. The firmness of plant-based yogurt from oat and chickpea.

Ratio (% Oat : % Chickpea)	Firmness(N)
5 : 5	1.15 ± 0.08^{c}
6 : 6	1.85 ± 0.09^{b}
7 : 7	4.93 ± 0.03^{a}

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same column are significantly different ($p \leq 0.05$).

Both oat and chickpea are abundant in starch in their seeds accounting for 50% and 29- 46%, respectively, of dry weight (Punia *et al*., 2020; Hoover *et al*., 2010). During plant mixture pasteurization (72°C in this study), oat starch underwent gelatinization at 59.0-64.6°C(Schnitzenbaumer & Arendt, 2014). Simultaneously, the gelatinization process for chickpea starch occurred close to 71.7°C (Edwards *et al*., 2015). This process resulted in swelling and subsequent bursting of starch granules increasing the viscosity and thickening the mixture. Therefore, the amount of plant flour in the recipe considerably affected the final texture of yogurt. The greater quantity of plant starch contributed to yogurt with increased viscosity, thicker and firmer texture. Furthermore, the amount of protein in the raw materials has been shown to play an importance role in yogurt texture. As the fermentation proceeded, lactic acidaccumulated by the yogurt culture, LAB, reduced the yogurt pH causing the proteins to precipitate and rebind to form gel-like coagulums (Shin *et al*., 2021). In plant materials likeoat and chickpea, globulins are the major protein type comprising 50-80% of the seed protein (Anderson, 2014; Chang *et al*., 2011). During lactic acid fermentation of oat bran, the solubility of globulins decreased leading to the structural change and aggregation of globulins (Loponen *et al*., 2007). The formation of protein aggregates is known to be positively associated with the gel structure of the product. Hence, the firmer texture of yogurt could be partly explained by the presence of higher protein in the recipe.

From the evaluation of product acceptance (Table 2), yogurt produced from oat andchickpea at 6:6 ratio was favored by panelists in quality attributes of appearance, sweetness, and viscosity ($p \leq 0.05$). The viscosity level of yogurt was positively correlated with thefirmness of yogurt. As stated earlier, the 7:7 recipe seemed to bring about the overly firm texture. The 6:6 ratio yogurt was consequently received a higher rating in terms of viscosity.

In the following section of experiments, the proportion of 6% oat and 6% chickpea was chosen as the foundation for further modification of plant-based yogurt recipe.

Table 2. Sensory evaluation (9-point hedonic test) of plant-based yogurt from oat and chickpea.

Sensory attributes	Ratio (% Oat : % Chickpe		
	5 : 5	6 : 6	7 : 7
Appearance	5.60±2.07[a]	5.39±1.91[ab]	4.84±1.79[b]
Color[ns]	5.39±2.07	5.24±1.92	5.48±1.85
Aroma[ns]	5.24±2.19	5.39±2.12	5.48±2.14
Sweetness	4.96±1.97[b]	5.27±1.77[a]	4.78±1.94[b]
Sourness[ns]	4.84±1.98	5.18±1.82	4.96±1.91
Viscosity	5.06±1.88[b]	5.72±2.06[a]	5.42±2.09[ab]
Mouthfeel[ns]	4.66±1.93	4.96±2.15	5.09±1.90
Overall liking[ns]	5.45±2.12	5.51±1.87	5.30±2.14

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

Effect of the level of pea protein isolate substitution on the quality and acceptanceof yogurt

A major shortcoming of plant-derived foods concerns the low quantity of protein ascompared with animal-based foods. In order to formulate a high-protein plant-based yogurt, chickpea flour was partially replaced with pea protein isolate. With the fixed oat percentage of6%, four yogurt recipes were compared varying the amount of pulse components consisting ofchickpea and pea protein at concentrations of 6:0, 4:2, 3:3 and 2:4. The color measurement of yogurt incorporated with pea protein was performed (Table 3). At 4% pea protein, the lowest L* (65.49) and a* (3.41) values were obtained indicating the product color on the whiteness side with a hint of redness ($p \leq 0.05$). Regardless of pea protein addition, the b* (17.64-18.35) values were not statistically different showing the product color toward the darker shade of yellow ($p > 0.05$). As the proportion of pea protein increased, the firmness of yogurt became greater with the highest firmness of 1.97 N observed in the 4% pea protein recipe ($p \leq 0.05$).As discussed earlier, protein composition in starting raw materials was strongly related to formation of protein coagulums and structure of gel network during yogurt fermentation. The high coagulum strength was reported in lactic acid fermentation of 5% pea protein contributing to the increased viscosity of the sample (Shin *et al.*, 2021). The presence of pea protein in the recipe was likely responsible for the enhanced firmness of yogurt.

Chemical quality of plant-based yogurt was assessed (Table 4). The higher level of pea protein resulted in the yogurt with higher acidity, lower pH, and lower syneresis ($p \leq 0.05$). Syneresis is an undesirable characteristic of yogurt occurring when liquid is separated from the gel structure. Fermentation of 5% pea protein suspension revealed an almost absence of syneresis (Shin *et al.*, 2021). Pea protein exhibited strong gelling properties as pH gradually dropped due to lactic acid production. The gel formation also increased the viscosity of themixture and the water holding capacity reducing syneresis (Klost & Drusch, 2019).

Table 3. The color and firmness of plant-based yogurt partially substituted with pea protein.

Ratio	Color			Firmness(N)
(% Chickpea : % Pea protein)	L*	a*	b*ns	
6 : 0	66.97±0.23[a]	4.26±0.25[a]	18.35±0.49	1.68±0.12[b]
4 : 2	66.99±0.28[a]	3.82±0.07[ab]	17.99±0.30	1.75±0.09[ab]
3 : 3	66.02±0.21[ab]	3.52±0.07[b]	18.17±0.55	1.87±0.04[a]
2 : 4	65.49±0.18[b]	3.41±0.01[b]	17.64±0.50	1.97±0.05[a]

Oat was fixed at 6% proportion in all recipes. Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same column are significantly different ($p \leq 0.05$).

Column headers with ns superscripts indicate that values within the same column are not significantly different ($p > 0.05$).

The protein contents of yogurt substituted with 0, 2, 3 and 4% pea protein were 3.74, 5.58, 6.71 and 7.98%, respectively (Table 4). According to the regulation by the Ministry of Public Health of Thailand, the yogurt recipes with 3 and 4% pea protein can be legally claimed "highprotein" on the product label as they contain more than 10 g of protein for the serving size of 150 g (Notification of the Ministry of Public Health, 1998).

Table 4. The protein content, syneresis, pH and acidity of plant-based yogurt partially substituted with pea protein.

Ratio (% Chickpea : % Pea protein)	% Protein	% Syneresis	pH	% Acidity
6 : 0	3.74±0.06[d]	30.96±0.18[a]	4.45±0.02[a]	0.27±0.01[b]
4 : 2	5.58±0.05[c]	15.83±0.37[b]	4.37±0.20[b]	0.38±0.03[ab]
3 : 3	6.71±0.24[b]	11.52±0.20[c]	4.21±0.04[c]	0.45±0.05[a]
2 : 4	7.98±0.18[a]	7.81±0.82[d]	4.15±0.03[d]	0.48±0.02[a]

Oat was fixed at 6% proportion in all recipes. Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same column are significantly different ($p \leq 0.05$).

The sensory assessment of yogurt with pea protein composition was conducted using 30 untrained panelists familiar with yogurt consumption (Table 5). Yogurt with 3% pea protein was preferred in more quality attributes comprising color, sweetness, sourness, mouthfeel, andoverall liking ($p \leq 0.05$). For plant-based yogurt production, 3% pea protein was chosen as the most suitable dosage considering the high-protein content and positive effects on manyproduct quality aspects. Nevertheless, the overall acceptability score of 6.25 was not very high and categorized as "like slightly". It was commented to be because of the product's beany flavor. In addition, LAB count of 3% pea protein yogurt was 8.26 log CFU/g (Table 6) exceeding the minimum requirement (6-7 log CFU/g) for the number of live microorganismsin the final product to ensure health benefits from consumption (Kurman & Rasic, 1991).

Table 5. Sensory evaluation (9-point hedonic test) of plant-based yogurt partially substituted with pea protein.

Sensory attributes	Ratio (% Chickpea : % Pea protein)			
	6 : 0	4 : 2	3 : 3	2 : 4
Appearance	5.71±1.74[b]	5.81±1.42[ab]	6.15±1.66[ab]	6.18±1.65[a]
Color	5.65±1.57[b]	6.06±1.43[ab]	6.25±1.68[a]	6.12±1.60[a]
Aroma[ns]	5.75±1.77	5.96±1.67	5.96±1.71	5.84±1.70
Sweetness	5.62±1.62[ab]	5.28±1.72[b]	5.84±1.48[a]	5.71±1.51[ab]
Sourness	5.40±1.75[ab]	5.12±1.49[b]	6.12±1.46[a]	5.75±1.70[ab]
Viscosity[ns]	6.03±1.59	5.96±1.35	5.90±1.72	6.00±1.50
Mouthfeel	5.62±1.77[ab]	5.06±1.68[b]	5.93±1.93[a]	5.81±1.55[a]
Overall liking	5.81±1.67[ab]	5.43±1.58[b]	6.25±1.68[a]	5.78±1.64[ab]

Oat was fixed at 6% proportion in all recipes. Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

Table 6. Lactic acid bacteria (LAB) count of plant-based yogurt partially substituted with pea protein.

Ratio (% Chickpea : % Pea protein)	LAB count(log CFU/g)
6 : 0	7.64±0.15[b]
3 : 3	8.26±0.13[a]

Oat was fixed at 6% proportion in all recipes. Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same column are significantly different ($p \leq 0.05$).

Conclusion

The combination of 6% oat, 3% chickpea, and 3% pea protein isolate was the most appropriate ingredient ratio for developing a novel plant-based yogurt with a high-protein content (more than 10 g/150 g serving size) and a balanced amino acid composition. The developed yogurt is a soy-free and dairy-free alternative for people with soy allergy, lactose intolerance and cow's milk protein allergy. However, the organoleptic properties of plant- based yogurt should be improved in future studies to reduce the beany flavor by attempting new raw materials or processing techniques to obtain a higher product acceptability byconsumers. Additionally, prebiotic fibers should be supplemented to create a synbiotic yogurt with added health benefits.

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