Physicochemical and microbiological properties of Pudding Powder as a complementary food during storage

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Abstract. The product evaluation during storage is important because it is related to food safety. The pudding powder as a complementary food was made from skim milk powder, sugar, mocaf, and carrageenan which enriched with inulin and fortified with micronutrient premix. The pudding powder were packed in polyethylene plastic (PE) and aluminium foil (AF) pouches to evaluate the physicochemical and microbiology properties at 15°C, 30°C, and 45°C for 4 weeks. The data were analyzed using IBM SPSS Statistics 20 and analysis of variance (ANOVA) with Duncan’s means test (significance level of p<0.05). The moisture content, protein, and lightness of all pudding powder were significantly decreased, while a value and Total Plate Count (TPC) were significantly increased after storage. The storage with AF pouches were not significantly affect the weight of pudding powder after first week storage. The water activity (a_w) of AF45 was the highest among others. It affects the decreasing of protein, increasing of TPC, browning and caking on this pudding powder. The best storage condition of pudding powder for both pouches was at temperature 15°C.

1. Introduction
The inulin-enriched pudding powder for complementary food has been developed to support the growth and development of children over one year old [1], [2]. The sensory evaluation assessment of pudding as complementary food showed a good response from both mother and children’s under 1-5 years old [1]. However, the iron and zinc contents did not meet the SNI requirement for instant complementary food powder [1]. Thus, the product development has been carried out to increase mineral content, especially iron. The iron and zinc content of pudding powder has been increased by tripled with mineral fortification which contain 50 mg/kg of iron, 30 mg/kg of zinc, 4 mg/kg of vitamin B2, 2.5 mg/kg of vitamin B1, and 2 mg/kg of folic acid [2]. The physicochemical properties of pudding powder with mineral fortified as a complementary food were 4.40% of moisture content, 1.00% of ash, 15.74% of protein, 0.12% of fat, 78.74% of carbohydrate, 419.13 mg/100mg of calcium. 2.04 mg/100g of iron, 7.18 mg/100g of zinc, 71.65 of lightness, -0.35 of a value, 11.05 of b value, and 105 cP of viscosity [2].

The complementary food must meet the nutritional needs and it is safe for infants. The safety of complementary food is related to contamination of chemical, metals, and microbial during preparation process and storage. Evaluation of the physicochemical and microbiological properties of the product during storage is essential as it is related to food safety and consumer acceptance. Moisture content and total microbes are the important characteristics of product during storage that related to food safety.
Furthermore, the color properties are essential characteristics of the product during the storage period that relate to consumer acceptance.

The research on the evaluation of physicochemical and microbiological properties in a complementary food during storage has been carried out. The effect of instant baby food storage has been done on the moisture content, peroxide value, and free fatty acids value, which were kept in double polyethylene bag at 20-25°C for 3 months [3]. Danarsi et al. [4] had conducted the effect of storage of instant porridge baby with the snakehead fish and pumpkin flour substitution on microbiological quality that stored in airtight plastic bag at 15°C for 4 weeks. The evaluation on the physicochemical characteristic of liquid and powder infant formula that stored at 10-40°C for 6 months had also been carried out [5], [6]. The storage affects the moisture content, color properties, surface protein, and total microbial of the complementary food [3]–[6].

The factors affect the physicochemical and microbiological properties changes during storage are type of packaging, storage temperature, relative humidity, and storage time. In this study, the pudding powder were packed in polyethylene plastic (PE) and aluminium foil (AF) pouches then stored at 15°C, 30°C, and 45°C for 4 weeks. The physicochemical and microbiological characteristics of powder pudding during storage were evaluated in this study. The evaluation was including moisture content, weight, protein, water activity, color properties, and total plate count.

2. Material and Method

The materials used in this study were skim milk powder from local store in Bandung, mocaf from Indomocaf in Bandung, sugar from local market in Subang, carrageenan, Orafti inulin (Synergy1-Oligofructose), and micronutrient premix SNI Enrichment Mix from Muhlenchemie.

2.1. Preparations and Storage Conditions of CF Pudding Powder

The pudding powder was prepared according to Kristanti et al. [2]. The pudding powder were packed in polyethylene plastic (PE) and aluminium foil (AF) pouches then stored at 15°C, 30°C, and 45°C for 4 weeks. The physicochemical and microbiological properties evaluated were moisture content, water activity (aw), weight, protein, color properties, and total plate count (TPC).

2.2. Physicochemical Properties Analysis

The moisture content of pudding powder was analysed by the gravimetric method [7]. The water activity (aw) of pudding powder was determined by Smart Water Activity Meter HD-3A with WSC-4 water activity sensor. The weight of pudding powder was determined using analytical balance (FV-220C). The protein content was analysed by the Dumas combustion method using DuMAster Buchi D-480, Switzerland. The color of CF pudding powder was determined by Chromameter Konica Minolta CR-400 using CIE method. The color scales of CIE were estimated as: L = lightness 0 - 100 is white color; a = red color 0 – 60 and green color 0 – (-60); b = yellow color 0 – 60 and blue color 0 – (-60). The total plate count (TPC) method was evaluated by AOAC [7]. The sampling and analysis were done every week during storage time.

2.3. Statistical Analysis.

Three replications of analysis from pudding powders were used in all of the quality measurements. The data were analyzed using IBM SPSS Statistics 20 and analysis of variance (ANOVA) with Duncan’s means test (significance level of p<0.05). All data were presented as the mean with standard deviation.

3. Result and Discussion

The physicochemical properties of pudding powder as complementary food in the initial observation (week 0) were 3.67% of moisture content, 15.50% of protein, 0.42 of water activity (aw), 32.16 g of weight powder, 73.62 of lightness, 0.18 of a value, 11.08 of b value, and 0.92x10⁴ CFU of total plate count. These results are slightly different from our previous studies. The physicochemical properties of pudding powder as a complementary food were 4.40% moisture content, 15.74% of protein, 71.65 of
lightness, -0.35 of a value, and 11.05 of b value [2]. The differences in physicochemical properties in pudding flour occur due to the different source of mocaf used in the formulation.

The moisture content of pudding powders was significantly decreased during storage in 4 weeks, except for PE30 which was increased. The PE45 sample was the pudding powder that had a highest decline in moisture content, it formed an agglomerate. The moisture content of pudding powder during storage was presented in Figure 1. The moisture content of spray dried yogurt powder and mango soy fortified yogurt powder stored in aluminium limited polyethylene and high density polypropylene pouches were increase during storage time [8], [9]. The moisture content of instant baby food was increase during storage time at 20-25°C in double polyethylene bag [3]. Other research showed that moisture content spray dried camel milk powder was slight decline during storage time in plastic petri dish polystyrene at relative humidity (RH) levels of 11%, 22%, and 32% [10]. The research of Masum et al. [6] showed that the storage of instant milk formula powder at 40°C for 15 day caused the very low of moisture content and the powder was caked. The factors may contribute to moisture content decrease during storage are permeability of packaging, storage temperature, and storage time.

![Figure 1. The moisture content of pudding powder as complementary food during storage](image)

The weight of pudding powder stored in aluminium foils were significantly increase in first week then it was stable until final week 4, except for AF45 sample that stable during storage period. Furthermore, the weight of pudding stored in polyethylene plastics were significantly increase during storage, except for PE45 sample which was drastically declined (Figure 2). These results study was not in accordance with previous study. The storage of dried persimmons at temperature of -20°C, 5°C, 12°C, and 25°C for 70 days was not affected by their weights and moisture contents [11]. The weight rising of PE15 and PE30 may cause by the increasing of moisture content during storage. The drastically decline of PE45 sample were due to drastically decrease of moisture content during storage (Figure 1).

The storage condition affects the water activity of pudding powder during storage (Table 1). The water activity of PE15, PE30, and AF45 significantly increased after storage. However, the $a_w$ of PE45, AF15, and AF30 were significantly decreased after storage. The increasing of the $a_w$ in AF45 was the highest among others. The changes in water activity of the pudding powder are caused by changes in moisture content during the storage period. The water activity of spray dried bovine colostrum powder stored in aluminium laminated polyethylene and polyethylene pouches increased during storage at
temperature 4°C, 25°C, and 50°C for 90 days [12]. The increasing of water activity in Yu et al. [12] study was accompanied by an increasing of moisture content. Other research showed that the storage of commercial goat milk powder and spray dried yogurt powder did not affect the water activity [8], [13].

The permeability of packaging affects the characteristics of food products during storage, especially moisture content and water activity. Plastics was relatively permeable to small molecules such as gases, water vapour, organic vapours, and liquids [14], but aluminium paper foil was not permeable for the oxygen [15]. The permeability of polyethylene was higher than the permeability of aluminium foil, there were 0.05 and 0.004 gH₂O/day.m².mmHg [16]. Generally, the high packaging permeability makes the characteristic of food products more easily change so that the shelf life is short.

![Figure 2. The weight of pudding powder as complementary food during storage.](image)

| Sample | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 |
|--------|--------|--------|--------|--------|--------|
| PE15   | 0.42±0.00³Ba | 0.42±0.00³Ba | 0.42±0.00³Bb | 0.44±0.01³Ab | 0.44±0.01³Ab |
| PE30   | 0.42±0.00³Ba | 0.40±0.00³Db | 0.41±0.00³Cab | 0.43±0.00³Ab | 0.43±0.00³Ac |
| PE45   | 0.42±0.00³Ba | 0.34±0.00³Cd | 0.34±0.01³Ce | 0.33±0.01³De | 0.35±0.01³Ee |
| AF15   | 0.42±0.00³Ab | 0.40±0.00³Cb | 0.40±0.00³Cc | 0.41±0.00³Bc | 0.40±0.00³Cd |
| AF30   | 0.42±0.00³Ba | 0.39±0.00³Cc | 0.39±0.00³Cd | 0.39±0.01³Cd | 0.39±0.00³Cd |
| AF45   | 0.42±0.00³Ca | 0.40±0.03³Eb | 0.48±0.00³Ba | 0.48±0.00³Ba | 0.49±0.00³Ac |

Values are expressed as mean ± standard deviation. Means in the same column (lowercase letters) and row (uppercase letters) with different letters were significantly different at p < 0.05.

The protein is one of the parameters that can be affected by temperature changes during storage. The protein of pudding powder during storage showed in Figure 3. The protein content of PE15 sample was relative stable until the second week of storage. It was decreased after the third week storage. Other protein samples were significantly declined after four-week storage. Furthermore, the highest decline of protein after four-week storage occurred in AF45. The decrease in protein content in the pudding powder is probably caused by Maillard reaction or protein denaturation during storage. The surface protein content of infant milk formula powder was decline after storage at 22 °C and 40 °C for 180 days [6]. The denaturation protein enthalpy (ΔH) of dried egg white was decrease after storage at 25 and 40 for four months that indicated a partially unfolded protein conformation [17].
The lowest protein occurred in AF45 sample. The water activity of the pudding powder may play a role in protein damage. The AF45 sample had a highest water activity, which is 0.49 (Table 1). The high water activity affects the loss rate of amino acids such as lysine when the storage temperature above the glass transition temperature [18]. The storage of infant milk formula powder decreased the glass transition temperature that affect the increasing of caking [6]. The research of Masum et al. [6] showed that the glass transition temperature of infant milk formula powder in the initial observation (week 0) was 70-80°C then decline to 10-30°C and <0°C in storage conditions at 20°C and 40°C, 54% RH for 90 days, respectively.

Figure 3. The protein of pudding powder as complementary food during storage

The color properties of pudding powder as complementary food present in Table 2. The lightness of pudding powder was significantly decreased in all sample. The lightness in final observation were not significantly different. The a value of the PE pouches samples increased, while the AF pouches decreased, but the value were not significantly different. The b value of PE15, PE30, and AF15 significantly decrease, but it increased for AF30 even though it was significantly different, and the AF45 was significantly increased. The results of previous studies stated that the lightness of mango soy fortified yogurt powder and liquid infant formula were decreased, while the a and b value were increased after storage [5], [9]. The research study of Ho et al. [10] showed that the storage affect the increasing of b value and the decreasing of a value, but not affect the lightness of spray dried camel milk powder. The storage caused the increasing of color different, b value, and Maillard Browning Index (MBI) value in the high protein powder products [8], [12], [17], [19]. Other research showed that the lightness and a value of mango milk powder were increased, while the b value was decreased [20].

The factors affect the lightness of food product during storage are type of packaging, storage temperature, sugar and protein content of product, water activity, and storage time. The lowest of lightness and highest of b value were occur in the AF45 sample. This result indicated that the AF45 sample was darker and yellowish colored. Moreover, the AF45 was caked. The pudding powder had a browning reaction during storage period. The discoloration of pudding powder during storage lead by reaction between sugar and amino acids, its browning reaction of the Maillard reaction effect. Furthermore, water activity of the pudding powder also plays a role in Maillard reaction. The water activity contributes for the development of browning via non-enzymatic reaction, its Maillard reaction [21]. The high-water activity of product leads browning reaction. The AF45 sample was 0.49 (Table 1). The decreasing of the glass transition temperature during storage period of infant milk formula powder caused caking [6].
Table 2. The color properties of pudding powder as complementary food during storage

| Sample | Week | 0   | 1   | 2   | 3   | 4   |
|--------|------|-----|-----|-----|-----|-----|
|        |      | 0.14Bb | 0.40Aa | 0.02Bb | 0.24Da | 0.34Cb |
| PE15  |      | 73.77 | 81.54 | 71.17 | 71.57 | 72.40 |
| PE30  |      | 73.30 | 80.69 | 71.74 | 70.19 | 72.31 |
| PE45  |      | 72.69 | 80.95 | 71.74 | 70.60 | 72.33 |
| AF15  |      | 74.04 | 81.55 | 71.30 | 70.79 | 72.65 |
| AF30  |      | 74.18 | 81.19 | 70.32 | 70.67 | 72.33 |
| AF45  |      | 73.74 | 81.19 | 70.17 | 67.91 | 68.69 |

L Values are expressed as mean ± standard deviation. Means in the same column (lowercase letters) and row (uppercase letters) with different letters were significantly different at p < 0.05

The total plate count (TPC) in all pudding powder samples significantly increased after four-week storage (Figure 4). The TPC of pudding powder stored at temperature 15°C and 30°C in both polyethylene and aluminium pouch were relatively stable until third week storage. Furthermore, the TPC of these pudding powder in the fourth week storage were significantly increased. However, the TPC of AF45 sample particularly increased in the third week storage. In addition, the TPC of PE45 sample since the first week storage and continued to drastically increase until the fourth week storage. The TPC at the first week storage meet the SNI requirement for instant complementary food powder, but at maximum limits.

Figure 4. The total plate count of pudding powder as complementary food during storage
These results were consistent with the previous study. The TPC of the snakehead fish and pumpkin flour in instant porridge as complementary food increased during storage in air tight plastic bag at 15°C for four weeks [4]. Another research showed that TPC of the mango milk powder decreased during storage in tin can, metalized polyester, 4-ply lamites, and polystyrene bag at 5°C and 15°C for 8 months [20]. The increasing of TPC during storage is probably caused by the high of water activity in pudding powder. The water activity of pudding powder in this study were in the range of 0.34 -0.49. The high value of aw affects the eminent probability of microbial growth, resulted in the increasing of total bacteria in pudding powder.

4. Conclusion
The storage of pudding powder in polyethylene plastic and aluminium foil pouch at 15°C, 30°C, and 45°C for 4 weeks affect the moisture content, weight, protein, water activity, color properties, and TPC. The moisture content, protein, and lightness of pudding powder in both polyethylene plastic and aluminium foil pouches were decreased, while the a value and TPC were increased after storage. The storage with aluminium foil pouches were not significantly affect the weight of pudding powder after first week storage. The storage of pudding powder in aluminium foil pouch at 45°C had a highest of water activity than others in which lead to a browning reaction during storage. The best storage condition of pudding powder for both polyethylene plastic and aluminium foil pouches were at temperature 15°C. Furthermore, it is necessary to conduct sensory evaluation during storage in order to determine the consumer acceptance level.

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