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PII: S2772-5022(26)00745-6
DOI: https://doi.org/10.1016/j.afres.2026.102407
Reference: AFRES 102407

To appear in: *Applied Food Research*

Received date: 9 June 2026
Revised date: 14 July 2026
Accepted date: 15 July 2026

Please cite this article as: Ratmawati Malaka , Nahariah , Wahniyathi Hatta , Farida Nur Yuliati , Kasmiati , Maynor Alberto Vargas Vargas , Fitri Armianty Arief , Zulfi Arianty , Rizky Widiyanty Kadir , Sadam Suliman Moh. Yousef , Adham M. Abdoe , Fatty Acid Profile, Physicochemical Properties, and Microstructure of Dangke Whey Evaporated Milk, *Applied Food Research* (2026), doi: https://doi.org/10.1016/j.afres.2026.102407

Highlights

- Dangke Whey Evaporated Milk is made from the use of whey as a by-product of dangke cheese making.
- There are 3 types of Whey Evaporated Skim Milk, namely Whey Evaporated Skim Milk (WESM), Whey Evaporated Chocolate (WEC), and Whey Evaporated Green Bean (WEGB).
- Whey Evaporated Skim Milk and Whey Evaporated Chocolate contain high levels of Monounsaturated Fa y Acid (MUFA) (14-18%), a type of oleic acid.
- Dangke Whey Evaporated milk has poten al for use in func onal foods because it contains high levels of an oxidants (304.67 to 360.00 µM/mL), Vitamin A, and Vitamin D3.

Fatty Acid Profile, Physicochemical Properties, and Microstructure of Dangke Whey Evaporated Milk

Ratmawati Malaka[1*], Nahariah[1], Wahniyathi Hatta[1], Farida Nur Yuliati[1], Kasmiati[2], Maynor Alberto Vargas Vargas[3], Fitri Armianty Arief [4], Zulfi Arianty[5], Rizky Widiyanty Kadir[6], Sadam Suliman Moh. Yousef[7], Adham M. Abdoe[8].

[1]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Indonesia

[2]Department of Social Economics, Faculty of Animal Science, Hasanuddin University, Indonesia

[3]Department of Food and Bioresources Chemistry, National Technological University, Costa Rica

[4]Faculty of Animal Husbandry, Papua University, Indonesia

[5]Technition of Milk Processing Biotechnology Laboratory, Faculty of Animal Science, Hasanuddin University, Indonesia

[6]Doctoral Student, Animal Science Study Program, Hasanuddin University, Indonesia

[7]Gadarif University, Sudan

[8]Department of Food Control, Benha University, Egypt

Corresponding author: *malaka_ag39@yahoo.co.id

Abstract

Whey, the liquid separated from curds during cheese making, contains high-value proteins such as α-lactalbumin, β-lactoglobulin, and lactoferrin. This study developed Dangke Whey Evaporated Milk (DWEM) as a value-added product from Dangke cheese whey and evaluated the effects of fortification with skim milk (WESM), cocoa powder (WEC), or mung bean extract (WEGB) at 1, 2, and 3%. All formulations were supplemented with 1% brown sugar, evaporated at 85°C for 4 h, and sterilized at 120°C for 15 min. Physicochemical properties, proximate composition, vitamins, antioxidant activity, fatty acid profiles, and microstructure were evaluated. The main technical improvement is the transformation of locally underutilized Dangke whey into a shelf-stable, low-sugar evaporated dairy matrix enriched with locally available ingredients. Antioxidant activity increased with fortificant concentration and ranged from 304.67 to 360.00 µM/mL, with the highest value in WEGB 3%. WESM 1% had the highest protein (7.5%) and fat (8.0%), whereas WESM 3% had the highest carbohydrate

(22.7%). Vitamin A was highest in WEGB 3% (246 µg), and vitamin D3 was highest in WEC 3% (85 IU). Viscosity ranged from 85 to 110 mPa·s, and pH ranged from 6.6 to 6.9. Fatty acid analysis indicated that DHA and EPA were not detected, whereas trace linoleic acid and α-linolenic acid were detected in some formulations. Microstructural observations showed distinct particle and fat globule distributions among WESM, WEC, and WEGB. These findings support DWEM as a potential circular dairy product; however, further validation using blank whey controls, complementary antioxidant assays, and higher-resolution microscopy is needed.

Keywords: whey, evaporated milk, fatty acid, omega-3. Omega-6

1. Introduction

Milk is a very important food ingredient in human life (Brilianty et al., 2022; Majumder et al., 2025). Animal protein is essential as a food source for human health and national resilience (Weindl et al., 2020). Dangke cheese whey (DCW) is the byproduct of its production (Faridah, 2019), which is a traditional cheese of Enrekang, South Sulawesi (Dirayanti et al., 2025), that still contains nutrients in the form of high-quality protein, namely α-lactalbumin, β-lactoglobulin, and lactoferrin (Malaka, 2014; Mehra et al., 2021), whose utilization is still not optimal because it is generally still wasted (Zandona et al., 2021). Several studies show that the average amount of whey wasted can reach 80-90 % of the total milk made into cheese (Besediuk et al., 2024). So, if milk production reaches 1000 liters/day, only about 300 liters become cheese, while the remaining 700 liters are whey.

Evaporated milk (EM) is milk from which some of the water has been removed and then heated in sealed cans at a temperature of at least 100°C for a period of time, removing 60% of the water from fresh milk, which gives it a creamy texture, pale tan color, and a long shelf life. EM is sterile, light-colored, and creamy. EM, also known as concentrated milk, is made by removing some of the water to a certain concentration. Approximately 6.7% of whole milk is concentrated, while the remainder is made from skim milk and buttermilk. This product has grown rapidly, particularly in canned sweetened evaporated milk (Malaka, 2014).

Commercially available EM contains too much sugar. Furthermore, its nutritional value still requires supplementation with solids, which is why it's often supplemented with skim milk. The addition of other ingredients, such as mung beans (green beans) or chocolate, still requires research and quality evaluation.

This study proposes a practical innovation for converting liquid Dangke cheese whey into Dangke Whey Evaporated Milk (DWEM), a concentrated dairy product designed for functional beverage applications. Compared with conventional evaporated milk based mainly on fresh milk and high sugar addition, the approach in this work uses an underutilized local

whey stream, applies a lower added-sugar level (1% brown sugar), and evaluates fortification with skim milk, cocoa powder, and mung bean extract. The technical improvement lies in combining controlled evaporation, sterilization, and local fortificants to increase solids, viscosity, micronutrient content, and antioxidant activity while reducing the environmental burden of whey disposal. The application advantage is therefore twofold: it supports circular dairy processing for smallholder Dangke producers and offers a basis for developing shelf-stable, value-added whey beverages that can contribute to animal-protein availability and food security in the animal protein sector can be increased to support the intelligence of children and the younger generation and achieve the international SDGs goal of ending hunger (zero hunger).

2. Materials and Methods

2.1 Source of whey and other ingredients

Whey was taken from a byproduct of Dangke cheese production (100 L) from Enrekang Regency smallholder farm, transported to the biotechnology milk processing laboratory, Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Indonesia, using a cooling unit car, and kept chilled at 5°C in a refrigerator throughout the study. The whey was filtered before use. Skim milk, granulated sugar, mung beans, and cocoa powder were made from natural, local ingredients.

2.2 Procedure for making Evaporated DWEM

DWEM was produced by evaporating Dangke cheese whey (DCW) at 85°C for 4 hours, then adding 1, 2, or 3% skim milk, brown sugar, and green beans, and sterilizing by autoclaving at 120°C for 15 minutes. The parameters measured from all treatments were: 1. Physical quality testing, including viscosity, color, and pH. 2. Chemical quality, including protein, fat, carbohydrate, vitamin, and mineral content. 3. Microstructure. 4. Fatty acid content. The research data were then analyzed by analysis of variance.

The procedure for making DWEM begins with preheating at 50°C for 30 minutes, followed by heating at 85 °C for 4 hours, but the product will be more sensitive to heat; it must be thicker to prevent fat globules from coalescing during storage. The next step is evaporation at 60°C, with stirring every 15 minutes to prevent creaming in the final product. After the DCW has thickened, it is immediately cooled, placed in heat-resistant packaging, sterilized in an autoclave at 120°C for 15 minutes, and then quickly cooled while shaking to prevent sediment from forming at the bottom of the packaging (Malaka, 2014).

2.3 Product quality measurement

2.3.1 Proximate Test

Proximate testing is a quantitative chemical analysis method used to determine the main nutrient (macronutrient) content in food, feed, or fishery products, including moisture, ash, protein, fat, and carbohydrates (Cortés-Herrera et al., 2021). This test is essential for assessing quality, nutritional value, and nutritional labeling (nutrition facts) (Md Noh et al., 2020).

Main Components of Proximate Tests: Moisture Content: Determines the percentage of water in a material; Ash Content: Measures total mineral content; Protein Content: Measures crude protein (using the Kjeldahl method); Fat Content: Measures crude fat (using the Soxhlet method).

2.3.2 Protein measurement (Kjeldahl methods)

The DWEM sample was destroyed by heating with concentrated sulfuric acid (H_2SO_4) and a catalyst. The digestion result was then distilled with sodium hydroxide (NaOH), producing ammonia, which was captured in boric acid. The distillate was then titrated with hydrochloric acid (HCl) to determine the nitrogen content.

The sample is distilled. The digestate is cooled, and sodium hydroxide (NaOH) is added to neutralize the acid and convert ammonium to ammonia gas (NH_3). The ammonia gas is then distilled off and captured by a receiving solution, usually boric acid (H_3BO_3). DWEM samples are heated in concentrated sulfuric acid (H_2SO_4) and a catalyst (K_2SO_4 and copper/selenium) at 340-420 °C. This process breaks down organic bonds and converts nitrogen to ammonium sulfate, resulting in a clear solution.

The resulting digestion solution is then titrated. The ammonia in the receiving solution is titrated with a standard acid solution (HCl or H_2SO_4) to measure the amount of nitrogen. The total nitrogen content is calculated and then multiplied by the protein conversion factor (generally 6.25 for food ingredients) to obtain the crude protein content.

The protein calculation formula is:

$$\%\,(N) = \frac{(V_{HCl} - V_{Blanco}) \times N_{HCl} \times 14.007}{\text{mg Sampel}} \times 100\%$$

$$\%\,Protein = \%\,N \times Conversion\ Factor \quad (6.38)$$

2.3.3 Fat Measurement (Soxhlet Methods)

The first step in this analysis is DWEM preparation, which involves drying the sample and then grinding it to a powder. After that, the sample was weighed to approximately 1 gram. The sample was placed into a filter cartridge with fat-free cotton. Next, a boiling stone was placed in a filter flask. The boiling stone was heated to 105°C, cooled in a desiccator, and then the filter flask was weighed. The filter cartridge was inserted into a Soxhlet apparatus and extracted with p.a. hexane. Next, the extractor was connected to a condenser. The filter flask was placed under a fat extractor (Fatex) to collect the crude fat extraction results. The filter flask was separated from the fat, then dried in a 105°C oven until a stable weight was obtained (± 4-6 hours). After that, the individual flask was cooled in a desiccator, and its final weight was measured. Crude fat content is obtained using the formula: a = weight of the filter flask and sample before the fat extraction process (g), b = final weight of the filter flask (g), x = sample material (g).

$$Crude\ fat\ = \frac{b-a}{x}\ x\ 100\%$$

a = weight of the filter flask and sample before the fat extraction process (g)

b = final filter flask (g)

x = material samples (g)

2.3.4 Lactose measurement

Approximately 1 g of the DWEM sample was weighed and transferred to a 100 mL volumetric flask. The sample was dissolved in a small amount of hot water, and the flask was diluted to the mark with distilled water. A 10 mL aliquot was pipetted into an Erlenmeyer flask, followed by 15 mL of distilled water and 25 mL of Luff-Schoorl's solution. The mixture was boiled for 10 minutes, then cooled. Next, 15 mL of 25% H_2SO_4 was added gradually to allow the foam to dissipate. Then, 10 mL of 20% KI was added. The mixture was titrated with 0.1 N sodium thiosulfate until a light yellow color appeared. Starch was added, and the titration continued until the blue color disappeared. The titration volume was recorded, and the lactose content was calculated (Portnoy & Barbano, 2021).

Ten milliliters of distilled water were pipetted into a flask, followed by the addition of 25 mL of Luff-Schoorl solution. The mixture was heated to a boil and maintained at a rolling boil for 10 minutes. After cooling, 15 mL of 25% H_2SO_4 was slowly added, and the mixture was allowed to stand briefly until the bubbles had dissipated. Subsequently, 10 mL of 20% KI was added, and the solution was titrated with 0.1 N sodium thiosulfate until a light yellow color was observed. Starch indicator was then added, and the titration was continued until the blue

color disappeared. The titration volume was recorded, and the lactose content was calculated.

2.3.5 Antioxidant Activity Test

Preparation of phosphomolybdate stock solution: 3.0 mL of sulfuric acid was added to 0.199 grams of sodium phosphate and 0.247 grams of ammonium molybdate (Hussen & Endalew, 2023). The mixture was diluted with distilled water to a final volume of exactly 50.0 mL. Determination of the maximum absorption wavelength: 1.0 mL of the DWEM solution was added to 1.0 mL of the phosphomolybdate reagent. The solution was incubated at 95°C for 60 minutes. Then, 5.0 mL of ethanol was added. The mixture was allowed to stand until the operating time was reached. The solution was measured using a UV-Vis spectrophotometer over the wavelength range 500–900 nm.

DWEM solutions were prepared at concentrations of 0.6, 0.7, 0.8, 0.9, and 1.0 mg/10 mL. Each solution was pipetted into a 1.0 mL volume, and 1 mL of phosphomolybdate reagent was added. The solution was heated at 95°C for 60 minutes. Then, 1 mL of the solution was taken, and ethanol was added to bring the total volume to exactly 5 mL. The mixture was measured for absorbance at its maximum wavelength using a UV-Vis spectrophotometer, and the measurement was replicated 3 times.

DWEM was weighed to an accuracy of 50 mg, then dissolved in ethanol to exactly 25 mL. Positive control stock solution and evaporated whey milk with the addition of skim milk (WESM), chocolate (WEC), and green beans (WEGB) were each pipetted into a test tube to a final volume of 1.0 mL, and then 1 mL of phosphomolybdate reagent was added. The solution was incubated at 95°C for 60 minutes. After incubation, ethanol was added to each solution to a final volume of exactly 5.0 mL. The mixture was measured for absorbance at its maximum wavelength using a UV-Vis spectrophotometer. Replication was carried out 3 times.

The antioxidant activity of the DWEM solution is expressed as mg quercetin equivalents per gram (mg QE/g). This parameter is obtained from a linear regression of absorbance versus quercetin concentration (y = bx + a), with y = sample absorbance and x = concentration. The x value obtained from this calculation is then entered into the formula.

$$\text{Antioxidant activity} = \frac{\text{concentration } \left(\frac{\text{mg}}{\text{mL}}\right) \times \text{Volume (mL)} \times \text{dilution factor}}{\text{mg sample} \times 1/1000}$$

2.3.6 Microstructural Examination (Phase-contrast Microscopy)

A drop of DWEM sample is placed on a slide. The phase plate, located on the objective lens, slows down the direct light and increases the contrast. The specimen, a suspension of

DWEM, is placed on the slide. Because the contrast is produced optically, the specimen does not need to be fixed or stained (which often kills cells). The phase contrast condenser is adjusted so that the light ring from the condenser matches (aligns) with the phase ring on the objective lens.

The microscope light is turned on, and the diaphragm is adjusted to achieve optimal light intensity. The specimen is focused. Microstructures in evaporated milk will appear in contrast (dark/light), even though the specimen is transparent. Images can be captured with the microscope camera for analysis. Images were acquired without spatially calibrated scale information. Therefore, scale bars could not be added retrospectively without introducing a risk of inaccurate size representation. The images are presented only for qualitative comparison of particle morphology, aggregation, and distribution among the DWEM formulations.

2.3.7 Fatty acid examination

All reagents and solvents used in this study were of HPLC grade. The materials used were 14% boron trifluoride (BF3), chloroform, pyrogallol, 2,2,4-trimethylpentane, undecanoic acid (C11:0), hexadecanoic acid (palmitic acid) (C16:0), octadecanoic acid (stearic acid) (C18:0), cis-9-octadecenoic acid (oleic acid) (C18:1), cis,cis-9,12-octadecadienoic acid (linoleic acid) (C18:2(n-6)), cis-11-vaccenic acid methyl ester, all-cis-7,10,13,16,19-docosapentaenoic acid methyl ester, and 37 mixed fatty acid methyl ester (FAME) standards. Ethyl ether, petroleum ether, hydrochloric acid (HCl), anhydrous sodium sulfate, and methanol were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Evaporated milk samples were stored at −70°C in a freezer before testing.

Fatty acids in the samples were methylated as esters, following the Association of Official Analytical Chemists (AOAC) method 996.06 (AOAC, 2010). A 1 mL evaporated milk sample was mixed with 50 mg pyrogallol and 2 mL of undecanoic acid (5 mg/mL in chloroform) as an internal standard. The mixture was hydrolyzed with 10 mL of 8.3 M HCl in a water bath at 70±5°C for 40 minutes, then extracted with 25 mL of ethyl ether and 25 mL of petroleum ether, and methylated with 2 mL of 7% BF3 (in methanol) at 100°C (in a heating block) for 45 minutes. The extract was then dehydrated over anhydrous sodium sulfate and prepared for analysis.

Fatty acids were analyzed using an Agilent 7890B GC (Agilent Technologies, Palo Alto, CA, USA) equipped with an FID and an SPTM-2560 capillary column (100 m length × 0.25 mm inner diameter × 0.2 μm film thickness, Supelco, Bellefonte, PA, USA). The oven temperature was held at 100°C for 4 min, increased to 240°C at 3°C/min, and then held at 240°C for 20 min. The injector and FID temperatures were 225°C and 285°C, respectively. One

microliter of extract was injected at a split ratio of 50:1. The carrier gas was nitrogen at a constant flow rate of 1 mL/min. FAMEs (Fatty Acid Methyl Esters) from the samples were identified by comparing their retention times and elution sequences with 37 mixed FAME standards (20 mg/mL, dissolved in isooctane), cis-11-vaxenic acid methyl ester (1 mg/mL, dissolved in heptane), and all-cis-7,10,13,16,19-docosapentaenoic acid methyl ester (1 mg/mL, dissolved in heptane). Each fatty acid was analyzed qualitatively by comparing its peak area with that of undecanoic acid in the GC chromatogram.

2.4 Statistical analysis

Data were expressed as mean ± standard deviation by descriptive statistics, and the feed samples were calculated as a percentage using the SPSS software (version 26). In addition, logistic regression analysis was conducted, and an odds ratio with 95% confidence intervals was used to test the relationship between predictors and expected or outcome variables. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussions

Whey, a by-product, is often discarded or added to cows' drinking water, so its utilization is not optimal, even though whey still contains nutritional value, including high levels of protein (Oktafiyanti et al., 2024) and immunoglobulins. Whey is a liquid separated as a residue during the production of cheese and casein. In other words, whey, or milk serum, is the liquid that remains after casein is removed. Whey is an important source of protein for human and animal consumption (Minj & Anand, 2020). On average, there are 120 million tons of whey products worldwide (Malaka, 2014), and only about 50% are utilized. Whey accounts for 80-90% of the total volume of milk when solids or curd are separated (Ergasheva et al., 2023), and it still contains 50% of the nutrients from natural milk, including protein, lactose, vitamins, and minerals (Zandona et al., 2021).

Naturally, approximately 1900 nutritional components are found in milk and whey proteins, which can be separated into two fractions: an insoluble fraction, α-lactalbumin, and a soluble fraction, β-lactoglobulin (Bonnaillie & Tomasula, 2012; Madureira et al., 2007; Mehra et al., 2021). Furthermore, whey from milk contains lactoferrin, which is very beneficial for health (Mirzakulova et al., 2025). Table 2 shows the average composition of whey's nutritional components.

Table 1. Average composition of liquid whey.

Composition	Whey (%)
Total Solids	6.5
Water	93.4
Milk Fat	0.05
Protein	0.55
NPN	0.18
Lactose	4.8
Minerals (Ca, P, Na, K, Cl, Lactic Acid)	0.5

Electrophoretic examination of α-lactalbumin shows that it contains covalent bonds with carbohydrate groups (Permyakov, 2020). The amino acid sequence of α-lactalbumin is similar to that of lysozyme. Bovine α-lactalbumin B and chicken egg white lysozyme have identical amino acid residues at positions 49 and 4, and their disulfide groups are located identically, namely at 6-120, 28-111, 61-77, and 73-91. β-lactoglobulin contains five cysteine residues per mole, of which four form disulfide bonds (Barbiroli et al., 2022; Rahaman et al., 2015). β-lactoglobulin is divided into two types: A and B (Meza-Nieto et al., 2007; Z. L. Wang et al., 2024). Type B is less heat-stable, whereas type A is stable across all temperatures. These two types can be separated by electrophoresis (Wu et al., 2024).

Transferrin (TF) is also commonly referred to as a blood plasma protein (Schmaier, 2020). Lactoferrin (LF) is produced not only by the mammary glands but also by the lacrimal, bronchial, salivary, renal, and endometrial mucosal glands (Kowalczyk et al., 2022). This component is also found in specific granules within heterophilic leukocytes (Actor et al., 2009). TF and LF each appear as a single, large polypeptide chain (Ashraf et al., 2024; Saio et al., 2014) with 600–700 peptide residues.

Table 2. Nutritional content of evaporated condensed milk

Nutritional Component of Evaporated Milk (%)	
Milk Fat	6.9±0.7
Dry Ingredient	30.2 ±2.6
Proteins	8.5±1.9
Minerals	2.1±0.3
Lactose	12.9±1.4

Table 2 presents the nutritional content of Sweetened Evaporated Milk (SEM). Generally, SEM contains 44% added sugar, which acts as a preservative (usually sucrose or a mixture of sucrose and dextrose). It is generally made from skim milk, though it can also be made from whole milk. On average, 2/3 of Unsweetened Evaporated Milk (UEM) is made from skim milk. According to international standards, SEM must contain less than 8.5% milk fat. UEM milk does not contain sugar.

When making EM, the sterilization temperature is a concern because it can cause coagulation. EM must be carefully monitored because it has an abnormal salt balance that facilitates coagulation when pH changes (Wu et al., 2024). Therefore, in this evaporation process, temperatures below 100°C are used to minimize damage to proteins and other nutritional components (Wang et al., 2024). EM is resistant to bacterial damage, but its quality can change due to naturally occurring chemical events (Featherstone, 2016). Common natural chemical changes in EM include darkening of the product, protein hydrolysis due to water, and the formation of calcium citrate crystals that adhere to the can walls and remain when the contents are poured through a small hole in the lid (Deysher & Webb, 1952). EM as a food source has advantages and disadvantages. The advantages are that the product is sterile, can generally be supplemented with additional nutrients such as vitamin D, is more economical, and does not require refrigeration. The downside is that when it is used as an ingredient for making cheese, the curd becomes soft due to the homogenization process, which causes the fat to spread evenly between the casein molecules, so it cannot be made into butter, and the taste changes from that of fresh milk.

3.1 Chemical Properties of DWEM

The protein measurement method in this study used the Kjeldahl method (Syahfitri et al., 2025). The basic principle of this measurement is to measure the total nitrogen in the milk sample, then multiply it by a conversion factor (for milk, 6.38) to obtain the protein percentage.

The Kjeldahl method is a standard chemical analysis for determining total nitrogen and crude protein levels in organic materials (food, feed, soil) through three main stages: digestion, distillation, and titration. This method converts organic nitrogen to ammonium sulfate using concentrated sulfuric acid and a catalyst, then releases ammonia, and finally titrates to calculate the nitrogen content.

The Soxhlet method is a standard, semi-continuous solvent extraction technique used to measure crude fat in food (Karuppuchamy & Campanella, 2026) and feed samples by dissolving fat in hexane over 4–18 hours. The sample is dried, ground, and placed in a thimble, where hot solvent vapors continuously wash over it, extracting lipids, which are then quantified

by weight after solvent evaporation.

The results of testing the protein, fat, and carbohydrate levels in evaporated milk made from whey are shown in Table 3. Whey evaporated skim milk (WESM) with 1%, 2%, and 3% skim milk powder added, contains 7.3%-7.5% protein. Meanwhile, whey evaporated chocolates (WEC) have a protein of 6.6–6.8%. Whey evaporated green beans (WEGB) have a protein of 1.3%. Generally, condensed/evaporated milk made from fresh milk has a protein content ranging from 8.0–8.6%. Therefore, Table 3 shows that the protein content is lower in whey-based evaporated milk (WBEM). This can be explained by the fact that whey protein differs from the protein in fresh milk, which contains casein. The proteins in whey are α-lactalbumin and β-lactoglobulin (Tonolini et al., 2021).

The fat content of WESM is 7.8-8.0%, while that of WEC is 7.3-7.5%, and WEGB has the lowest fat content, at 1.3%. In general, EM contains 6.8% fat when made from full-cream milk, but when made from skim milk, it has no fat. Milk fat is a perfect burning material. So milk fat is an important energy source (German & Dillard, 2006). Milk fat is economically important because milk prices are based on its fat content (German & Dillard, 2006; Gómez-Cortés et al., 2018). Milk fat has a greater capacity to form body fat compared to fat from vegetable sources, but does not play an important role in the formation of body fat reserves. This body fat is also called circulating fat or physiological fat. Milk fat also contains vitamins (Gómez-Cortés et al., 2018), so butter has quite a high nutritional value.

The carbohydrate content of WESM is 22.5–22.7%. WEC has a carbohydrate content ranging from 22.2–22.4%. WEGB has the lowest carbohydrate content, at only 8.55–9.3%. EM generally contains around 13.0–14.5% of carbohydrates. The carbohydrate in milk is lactose (Gambelli, 2017).

Table 3. Antioxidant activity, protein, fat, and Carbohydrate of WESM, WEC, and WEGB.

Sample	Antioxidant activity (µM/mL)	Protein (%)	Fat (%)	Carbohydrate (%)
WESM 1%	304.67	7.5	8.0	22.5
WESM 2%	315.59	7.4	7.9	22.6
WESM 3%	322.48	7.3	7.8	22.7
WEC 1%	315.00	6.8	7.5	22.2
WEC 2%	325.00	6.7	7.4	22.3
WEC 3%	335.00	6.6	7.3	22.4

WEGB 1%	339.99	1.3	0.21	8.55
WEGB 2%	350.00	1.3	0.20	9.00
WEGB 3%	360.00	1.3	0.18	9.3

Note: WESM (Whey Evaporated Skim Milk); WEC (Whey Evaporated Chocolate); WEGB (Whey Evaporated Green Bean)

Antioxidants are compounds or systems that can reduce the reactivity of free radicals and stop chain reactions that can damage macromolecules in the body (Oroian & Escriche, 2015). Free radicals are atoms or molecules that have one or more unpaired electrons in their outermost orbital and are highly reactive (Chandimali et al., 2025). Free radicals can react rapidly with other atoms to fill the unpaired orbitals (Phaniendra et al., 2015). If free radicals are not inactivated, their reactivity can damage all types of cellular macromolecules, such as carbohydrates, proteins, lipids, and nucleic acids. Molecules whose electrons are taken will become new free radicals.

One of the macromolecules most susceptible to free-radical attack is lipid. This macromolecular damage occurs when free radicals react with unsaturated fatty acids (PUFAs), ultimately causing lipid peroxidation. Lipid peroxidation is a chain reaction that provides a continuous supply of free radicals that initiate further peroxidation. Lipid peroxidation can cause degenerative diseases, such as cancer, coronary heart disease, diabetes, and other metabolic syndromes. Lipid peroxidation also often occurs in foods such as oils. Lipid peroxidation in food can lead to the formation of volatile decomposition products such as aldehydes and ketones, which cause rancid odors (Chandimali et al., 2025; Hussen & Endalew, 2023). Antioxidants can be obtained from compounds with antioxidant activity, including vitamins C and E, carotenoids (carotenes and xanthophylls), and polyphenols (flavonoids, phenolic acids, lignans, and stilbenes) (Hussen & Endalew, 2023). These compounds can be explored from natural sources, which are believed to be safer for health than synthetic antioxidants.

3.2 Physical Properties of DWEM

Table 4 describes the physical properties of functional DWEM. Modern colorimeters produce digital output that can be automatically converted to industry-standard color values such as the CIELAB (L*, a*, b*) or CIELCH (L*C*h*) systems. Colorimeter readings yield darker, more yellowish colors, as indicated by the average $L*$, $a*$, $b*$ values. The color of a product plays a crucial role in food selection. It is generally recognized that color is a key factor in consumer preferences (Fernández-Vázquez et al., 2011). Customers generally associate color with freshness, ripeness, attractiveness, and flavor. The lightness (L*) value of DWEM products ranges from 75 to 85.26. The colorimetric assessment of L* correlates with human perception,

with a value ranging from 0 to 100, where 0 is dark, and 100 is the lightest or whitest. The brightest WESM is 1% skim milk, with a brightness value of 85.26%, and the darkest is 3% chocolate, with a value of 75. The a* value is represented as a red-to-green range, where red indicates a positive value and green indicates a negative value. The resulting EM shows that, with the addition of skim milk, it has a negative value between -3.09 and -2, indicating that the EM tends to be closer to green than red. Conversely, the one with chocolate added (WEC) has a positive value, meaning it tends to be closer to red than to green. While the WEGB has a negative value, adding 3% green beans yields a value of 0, indicating the color is neither close to red nor green. The b* value is represented as a blue-to-yellow range, where yellow indicates a positive value and blue indicates a negative value. The b* value in the DWEM samples ranges from 17.13 to 37, indicating that all samples are closer to yellow than to blue.

Table 4. Physical Properties of DWEM with Different Supplementation

Sample	L*	a*	b*	Viscosity (mPa·s)	pH
WESM 1%	85.26	-3.09	17.13	85	6.8
WESM 2%	84	-2.5	18	90	6.7
WESM 3%	82.5	-2	19	95	6.6
WEC 1%	80.59	2.63	35.81	100	6.8
WEC 2%	78	3	36.5	105	6.7
WEC 3%	75	3.5	37	110	6.6
WEGB 1%	82	-1	25	95	6.9
WEGB 2%	80	-0.5	26	100	6.8
WEGB 3%	78	0	27	105	6.7

Note: WESM (Whey Evaporated Skim Milk); WEC (Whey Evaporated Chocolate); WEGB (Whey Evaporated Green Bean); L* (0 is dark and 100 is the lightest); a* (represented as a red-to-green range, where red indicates a positive value and green indicates a negative value); b*(represented as a blue-to-yellow range, where yellow indicates a positive value and blue indicates a negative value)

Viscosity is an important physical property, especially for fermented milk products, cheese, and EM. Viscosity is a measure of the thickness of a product defined as the ratio of shearing stress to shear rate, measured with a viscometer and expressed in mPa·s /second (centipoise, cp) (Walstra, 1990). Normal milk has a viscosity of 1.5-2.0 cp at 20 °C. Yogurt has a viscosity between 10 – 80 cp, while soft cheese has a viscosity between 200 – 500 cp (Malaka

et al., 2023). The viscosity of water is 1.005 cP. From Table 4, DWEM has a viscosity between 85 and 110 cp, with the highest viscosity in WEC 3% and WEGB 3%.

The pH of fresh milk ranges from 6.6 to 6.7 (Rosca et al., 2019). The pH will decrease due to the activity of phosphate, citrate, and protein buffers. Fresh milk has amphoteric properties, meaning it is both acidic and basic. Most of the acid found in milk is lactic acid. However, milk acidity can be caused by acidic compounds such as complex phosphate compounds, citric acid, amino acids, and carbon dioxide dissolved in milk. It is important to remember that the normal milk pH range of 6.5 to 6.7 is indeed small, but because pH is on a logarithmic scale, the actual difference reflects ion activity. pH of WESM, WEC, and WEGB ranges from 6.6 to 6.9. This means that adding skim milk, chocolate, or green beans does not change the milk's pH; it remains normal.

Table 5. Vitamin A and D3 Values of DWEM with the Addition of Chocolate (WEC) and Green Bean Extract (WEGB)

Sample	Vitamin A (µg)	Vitamin D3 (IU)
WESM 1%	150[a]	60[a]
WESM 2%	160[b]	66[b]
WESM 3%	170[c]	65[b]
WEC 1%	150[a]	75[c]
WEC 2%	170[c]	82[d]
WEC 3%	200[d]	85[e]
WEGB 1%	170[c]	70[f]
WEGB 2%	220[e]	77[c]
WEGB 3%	246[f]	82[d]

Note: different symbols in the same column indicate significant differences ($P<0.05$). WESM (Whey Evaporated Skim Milk); WEC (Whey Evaporated Chocolate); WEGB (Whey Evaporated Green Bean)

The vitamin A and vitamin D3 content in DWEM increases after the addition of chocolate (WEC) and mung/green beans (WEGB). There is a significant difference in WESM, with a vitamin A range of 150–170 µg/100 mL, compared to WEC. Similarly, WEC 1–3% vitamin A ranges from 150–200 µg/100 mL, and WEGB 1–3% ranges from 170–246 µg/100 mL. These values are much higher than the vitamin A value in whole milk, which is 78 µg/200 mL in whole cow's milk. This is because most of the vitamin A is dissolved in whey, where it

is bound to fat, which is the basic ingredient in making this evaporated milk. The contribution of vitamin A nutritional value as a beverage for adults is indeed necessary, considering the maximum vitamin A requirement for adults is 1,500 μg/day. EM is commonly used as a flavor enhancer in coffee drinks or as a stand-alone beverage, especially in areas where fresh milk is not readily available. Vitamin A intake should be neither excessive nor deficient. Vitamin A is essential for maintaining eye health, boosting the immune system, supporting cell growth and development, maintaining healthy skin, and also acting as an antioxidant.

Vitamin D3 in condensed milk (Table 5) in WESM 1-3% is 60-66 IU, while with the WEC 1-3%, the vitamin D3 value ranges from 75-85 IU; likewise, with the WEGB 1-3%, the vitamin D3 value ranges from 70-82 IU. Vitamin D3, also commonly called cholecalciferol, is very important for bone health and immunity. Generally, commercial milk products contain 100-400 IU of vitamin D3 per liter, or about 100 IU per 178-250 mL, thus helping achieve 25% of the daily nutritional adequacy rate.

3.3 Microstructure

The microstructure of DWEM was observed using a phase-contrast microscope. Phase-contrast microscopy is suitable for observing transparent and unstained food systems because it converts differences in refractive index and specimen thickness into differences in image contrast (Yin et al., 2012). In milk-based systems, this technique allows visualization of dispersed structures such as fat droplets, protein-associated particles, and other suspended components without staining (Bihola et al., 2026). Therefore, this method was used to evaluate the general structural differences among the DWEM formulations.

Figure 1 shows the microstructure of DWEM samples prepared from Dangke whey. In the WESM sample, observed at 40× magnification (Figure 1A1), the dispersed components were less clearly distinguished. At higher magnification (Figure 1A2), spherical droplets became more visible and appeared to be distributed throughout the field of view. These spherical droplets were considered to be consistent with milk fat globules based on their morphology and the reported micrometer-scale size range of milk fat globules (Huppertz & Chia, 2021). Small granular structures were also observed among the droplets. However, because phase-contrast microscopy cannot definitively distinguish casein micelles or other protein-associated structures, these particles are described cautiously as colloidal or protein-associated structures. The relatively even distribution of these components suggests a more homogeneous structure, which may contribute to better physical stability and smoother product consistency.

In the WEC sample, observed at 40× and higher magnification (Figure 1B1 and B2),

the microstructure was clearly different from that of WESM. Spherical droplets consistent with fat globules were observed among larger chocolate-derived particles. At higher magnification, the chocolate particles appeared as larger clustered structures, with some granules connected to one another and forming chain-like aggregates. This aggregation may increase the structural complexity of the condensed system and contribute to higher viscosity, thicker texture, or reduced flowability. However, excessive particle aggregation may also reduce structural uniformity and influence product stability during storage.

The WEGB sample containing mung bean extract showed another distinct microstructural pattern (Figure 1C1 and C2). At 40× magnification, spherical droplets were less visible because the field of view was dominated by larger mung bean-derived particles. At higher magnification, the mung bean particles appeared as rounded granules and lobed clusters, with spherical droplets dispersed among them. The presence of these larger plant-based particles may increase the solid content and promote a denser microstructure. This structure may help improve body and consistency, but large or aggregated particles may also contribute to a less uniform texture if they are not evenly dispersed.

Overall, the microstructural differences among the DWEM samples indicate that the added ingredients influenced the arrangement and distribution of dispersed particles. A more uniform dispersion of spherical droplets and colloidal particles may be associated with better physical stability and smoother texture, whereas larger particle clusters or aggregates may increase viscosity and body but can also promote heterogeneity. Thus, the observed microstructure provides a possible mechanistic explanation for the macroscopic properties of the DWEM samples, particularly differences in texture, consistency, viscosity, and physical stability. Further confirmation using confocal microscopy or scanning electron microscopy is recommended in future studies to distinguish specific milk components more accurately.

3.4 Fatty Acid Profile of Evaporated Milk Whey

The fatty acid profile is very important in assessing the quality of the functional product to be produced. Saturated fatty acids (SFA), such as palmitic and stearic acids, are often avoided in functional products. Of the three DWEMs, the one with the highest palmitic acid content, at 35.57%, is WEGB. Meanwhile, the stearic acid content in all three DWEMs is less than 20%, which is the limit to be avoided. Monounsaturated fatty acids (MUFAs), namely oleic acid, which is good for heart health, are quite high in WESM (16.36% and 14.20%) and in WEC (14.90% and 17.93%).

The fatty acid to avoid in food is Trans Fatty Acid (TFA), specifically elaidic acid, which should not exceed 2% of the total dietary intake. In these DWEMs, this content is very

low, less than 0.3% (between 0.1 and 0.2%).

Unfortunately, these DWEM do not contain Docosahexaenoic acid (DHA) or Eicosapentaenoic acid (EPA; C20:5 n-3). Meanwhile, another Omega-3 element, α-linolenic acid C18:3 n-3, was detected in WESM and WEC at levels of less than 1% and was not detected in WEGB.

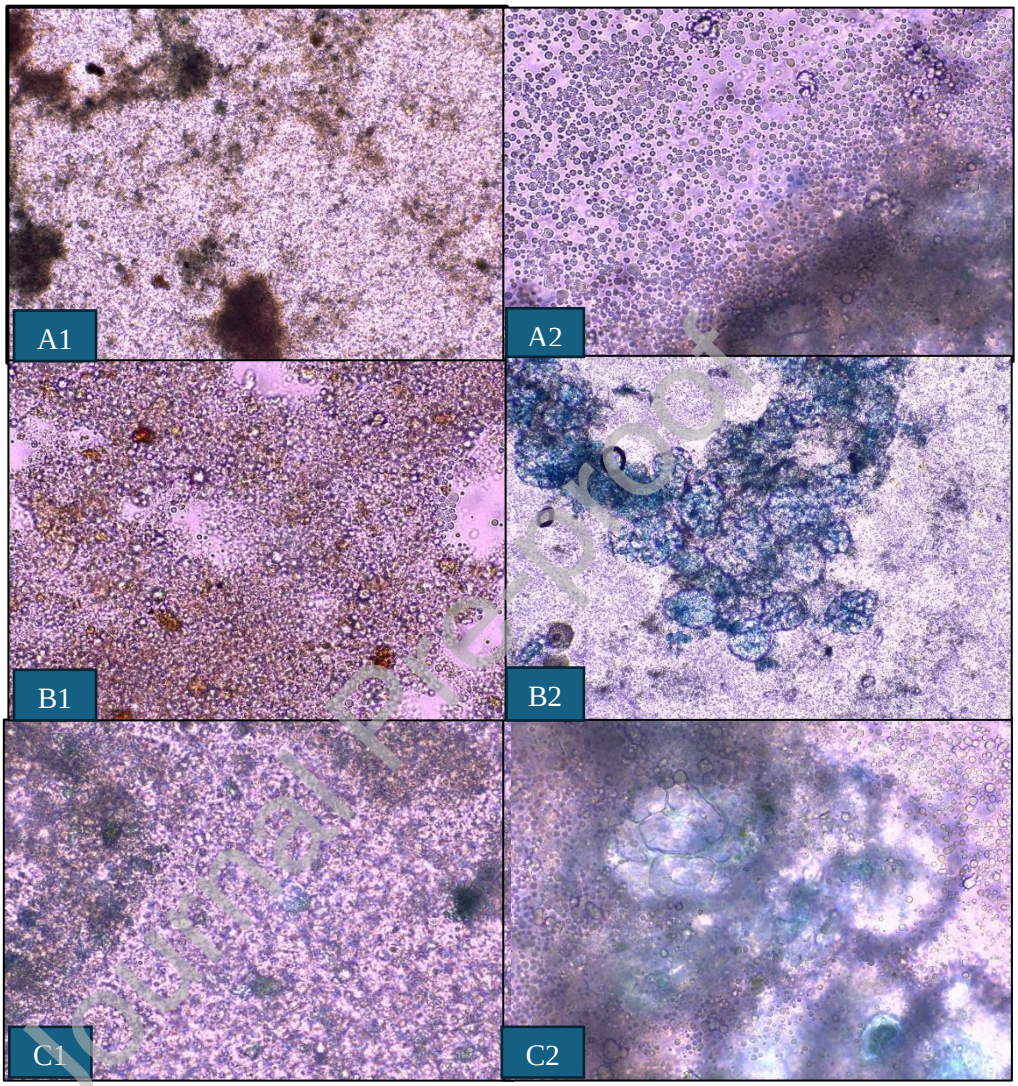

Figure 1. Microstructure of DWEM observed using phase-contrast microscopy: A1, WESM at 40 × 10 magnification; A2, WESM at 100 × 10 magnification; B1, WEC at 40 × 10 magnification; B2, WEC at 100 × 10 magnification; C1, WEGB at 40 × 10 magnification; and C2, WEGB at 100 × 10 magnification. Images were acquired without spatial calibration; therefore, scale bars are not provided, and the images should be interpreted qualitatively.

The fatty acids of WESM are butyric acid, caproic acid, caprylic acid, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, myristoleic acid, pentadecanoic acid, cis-10-pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic

acid, and elaidic acid. Linoleic acid, arachidonic acid, v-linoleic acid, Cis-11-Eicosenoic acid, behenic acid, tericosanoid acid, lignoceric acid. Fatty acids that were not detected in WESM were v-linoleic acid, cis-8,11,14-eicosenoic acid, cis-13,16-docosahexadienoic acid, enuric acid, cis-11,14,17-eicosatrienoic acid, cis-13,16-docosahexadienoic acid, cis-5,8,11,14,17-eicosapentaenoic acid, nerponic acid, and cis-4,7,10,13,16,19-docosahexaenoic acid. WESM had dominant fatty acids, namely oleic acid at 16.36%, followed by stearic acid at 5.89%, myristic acid at 3.45%, lauric acid at 1.25%, and capric acid at 1.09%.

Table 6. Fatty Acid Value of Sweetened Evaporated Milk Whey with the addition of skim milk powder.

No	Fatty Acid Composition	Results (w/w) on fat	% FA to % TFA area*	Results (w/w) on fat	% FA to % TFA area*	Results (w/w) on fat	% FA to % TFA area*
		WESM		WEC		WEGB	
1.	Butyric Acid C4:0	0.7183	1.8854	0.4700	1.7106	0.4305	0.9448
2.	Caproic Acid C6:0	0.7754	3.3911	0.5590	3.3901	0.6356	2.3240
3.	Caprilic Acid C8:0	0.5762	3.0400	0.4089	2.9912	0.5933	2.6171
4.	Capric Acid C10:0	1.0918	6.3746	0.8062	6.5268	0.9199	4.4902
5.	Undecanoic acid C11:0	0.0156	0.0968	0.0082	0.0711	0.0195	0.1012
6.	Lauric Acid C12:0	1.2474	6.7359	0.9227	6.9085	1.0176	4.5936
7.	Tridecanoid acid C13:0	0.0469	0.2819	0.0373	0.3110	0.0517	0.2598
8.	Myristic acid C14:0	3.4899	19.3846	2.8070	21.6191	3.1223	14.4994
9.	Myristoleic acid C14:1	0.2560	0.6048	0.2019	0.6615	0.0997	0.1970
10.	Pentadecanoic acid C15:0	0.6683	3.8333	0.5909	4.6998	0.6365	3.0524
11.	Cis-10 Pentadecanoid acid C15:1	0.0030	0.0076	0.0025	0.0090	Nd**	Nd**
12.	Palmitic acid C16:0	0.1660	0.8695	1.409	10.2501	8.1070	35.5703
13.	Palmitoleic acid C16:1	0.3134	0.6173	0.2715	0.7413	0.1242	0.2044
14.	Heptadecanoic acid C17:0	0.4451	2.5187	0.3918	3.0740	0.4124	1.9508
15.	Cis-10-heptadecanoid acid C17:1	0.0916	0.1860	0.0807	0.2271	0.0345	0.0585
16.	Stearic Acid C18:0	5.8897	27.5081	1.1177	7.2381	4.9588	19.3629
17.	Elaidic Acid C18:1n9c	0.0744	0.2194	0.1304	0.5332	0.0110	0.0270
18.	Oleic Acid C18:1n9c	16.3591	14.2022	14.8971	17.9325	3.2310	2.3451
19.	Linolelaidic acid C18:1n9t	0.1702	0.3211	0.7050	1.8445	0.0912	0.1438
20.	Linoleic acid C18:3n6	0.5239	1.0422	0.7666	2.1146	0.4908	0.8162
21.	Arachidic acid C20:0	0.1110	0.4941	0.1087	0.6656	0.1194	0.4408
22.	v-Linoleic acid,	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**

	C18:3n6						
23.	Cis-11-Eicosenoic acid C20:1	0.0407	0.0803	Nd**	Nd**	0.0138	0.0228
24.	Alfa-Linoleic acid, C18:3n3	0.0227	0.0556	0.0836	0.2838	Nd**	Nd**
25.	Heneicosanoic acid, C21:0	0.0225	0.1101	0.0163	0.1107	0.0227	0.0930
26.	Cis-11-Eicosenoic acid, C20:2	0.0042	0.0087	Nd**	Nd**	Nd**	Nd**
27.	Behenic acid, C22:0	0.0340	0.1419	Nd**	Nd**	0.0244	0.0850
28.	Cis-8,11,14-Eicosetrienoic acid, C20:3n6	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**
29.	Enuric acid, C22:1	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**
30.	Cis-11,14,17-Eicosatrienoic acid, C20:3n6	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**
31.	Tricosanoic acid, C23:0	0.0145	0.0625	Nd**	Nd**	0.0156	0.0566
32.	Arachidonic acid, C20:4n6	0.0027	0.0051	0.0199	0.0526	Nd**	Nd**
33.	Cis-13,16-Docosadienoic acid, C22:2	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**
34.	Lignoseric acid, C24:0	0.0130	0.0540	0.0134	0.0911	0.0184	0.0639
35.	Cis-5,8,11,14,17-Eicosapentaenoic acid, C20:5n3	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**
36.	Nervonic acid, C24:1	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**
37.	Cis-4,7,10,13,16,19-Docosahexaenoic acid, C22:6n3	Nd**	Nd**	Nd**	Nd**	Nd**	Nd**

Note: Nd (not detected)

The fatty acids contained in WEC include butyric acid, caproic acid, caprylic acid, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, myristoleic acid, pentadecanoic acid, cis-10-pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, elaidic acid, linolelaidic acid, linoleic acid, arachidonic acid, v-linoleic acid, tericosanoid acid, and lignoceric acid. Fatty acids not detected in WEC include v-linoleic acid, cis-11 eicosenoic acid, behenic acid, cis-8,11,14 eicosatrienoic acid, cis-13-16-doecosadienoic acid, enuric acid, cis-11, 14, 17 eicosatrienoic acid, tricoseasam, cis-13,16-docosadienoic acid, cis-5,8,11,14,17-eicosapentaeonic acid, nerponic acid, and cis-4,7,10, 13,16,19-docosahexaenoic acid. WESM had dominant fatty acids, namely oleic acid at 14.90%, followed by myristic acid at 3.42% and stearic acid at 1.12%.

The fatty acids contained in WEGB include butyric acid, caproic acid, caprylic acid,

capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, myristoleic acid, pentadecanoic acid, cis-10-pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, and elaidic acid. Linolelaidic acid, heneicosanoic acid, behenic acid, arachidonic acid, tericosanoid acid, and lignoceric acid. Fatty acids not detected in WEGB include cis-10 pentadecanoid acid, v-linoleic acid, linoleic acid, cis-11 eicosenoic acid, cis-8,11,14 eicosatrienoic acid, cis-13-16-doecosadienoic acid, enuric acid, cis-11, 14, 17 eicosatrienoic acid, arachidonic acid, cis-5,8,11,14,17-eicosapentaeonic acid, nerponic acid, and cis-4,7,10, 13,16,19-docosahexaenoic acid. WESM had dominant fatty acids, namely stearic acid at 4.93%, followed by oleic acid at 3.22%, myristic acid at 3.12%, and lauric acid at 1.02%.

4. Conclusion

This study demonstrates that Dangke whey can be developed into a value-added evaporated milk product with promising nutritional, antioxidant, physicochemical, and microstructural characteristics. The DWEM formulations exhibited distinct compositional profiles, indicating that the addition of skim milk, chocolate, and green bean ingredients influenced macronutrient content, vitamin levels, color, viscosity, and microstructure of the final products. WESM and WEC contained oleic acid as the dominant monounsaturated fatty acid, while omega-3 and omega-6 fatty acids were not detected in these formulations. All DWEM samples also showed relatively high antioxidant capacity in the phosphomolybdate assay, suggesting their potential as functional dairy-based products.

Beyond individual nutritional values, the findings indicate that DWEM formulations maintained pH levels close to those of fresh milk and exhibited acceptable viscosity and brightness. These properties are important for consumer acceptability, processing stability, and potential product development. Microstructural observations further support the differences among formulations, with dispersed fat globules, chocolate-derived clusters, and green bean particles contributing to the physical characteristics of each product.

Overall, the results suggest that Dangke whey, often considered a by-product, has strong potential as a base material for innovative dairy products. The development of DWEM may improve whey utilization, reduce dairy waste, and diversify functional milk-based foods. Future studies should further evaluate storage stability, sensory acceptance, bioactive compound profiles, and antioxidant activity using complementary assays to support the wider application and commercialization of DWEM products.

Aknowlegment

Thank you for funding research from the Team Research Group (TRG) at Hasanuddin University

Credit Author Contribution Statement

Ratmawati Malaka: Conceptualization, Methodology, Supervision, Project administration, Writing-original draft, Writing-review and editing.

Nahariah: Methodology, Validation, Supervision, Writing, Review, and Editing.

Wahniyathi Hatta: Methodology, Validation, Resources, Writing - review & editing.

Farida Nur Yuliati: Methodology, Data curation, Writing-review and editing.

Kasmiati: Investigation, Formal analysis, Data curation, Visualization.

Maynor Alberto Vargas Vargas: Validation, Formal analysis, Writing-review and editing.

Fitri Armianty Arief: Investigation, Data curation, Formal analysis.

Zulfi Arianty: Investigation, Data curation, Visualization.

Rizky Widiyanty Kadir: Investigation, Data curation, Formal analysis, Writing-review and editing.

Sadam Suliman Moh. Yousef: Validation, Writing-review, and editing.

Adham Moh. Abdoe: Validation, Writing-review and editing.

All authors have read and approved the final version of the manuscript.

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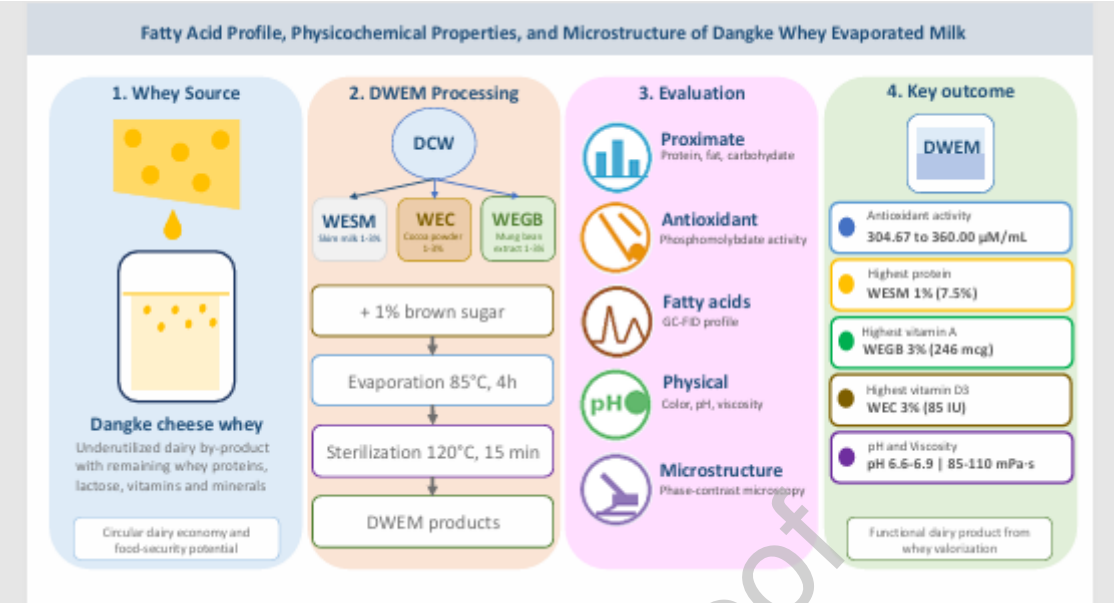
Graphical abstract



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.