# Optimized Blended Innovative Butter Comparative Study of Physicochemical, Functional and Sensory Properties with Whey Protein Addition

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**ABSTRACT**: This research aims to investigate the production of mixed butter of containing palm oil and natural butter and with the addition of whey protein concentrate (WPC) or milk permeate Moreover, the quality of butter was evaluated before and after and investigate its quality during storage at +5°C and - or -18°C for blended butter with similar physiochemical, functional, sensory, and rheological properties of butter-like recipes and improve its healthy and unhealthy value due to their content of saturated fatty acids and whey protein, which have functional and therapeutic properties. The results revealed that the pH values for samples stored at 5°C were less than those stored at -18°C. The replacement of milk permeate with whey protein concentrate resulted in slight changes in the free fatty acids (FFAs) values, specifically, the values were 0.257% and 0.285 % of the control and 100%WPC treatment, respectively. The melting point of blended butter was 38°C on the first day and 37°C after 6 months of storage, showing no significant differences between the control, WPC enriched samples, at different concentrations. of WPC. However, the total saturated fatty acid content of treatmented and control samples was higher than unsaturated fatty acids content; with values ranging from 51.5% and 52.32% and 45.60% and 45.34%, respectively. The hardness of samples stored at -18°C was greater than that samples stored at 5°C. The blended butter that contained 75% whey permeate recorded the highest rating, while general acceptability decreased for all samples after 6 months. Finally, microbiology tests showed that no live microorganisms were detected in all blended butter samples during the entire storage period.

**Keywords:** Spread Blended Butter, Butter-like recipes, Palm oil, Functional blended butter.

## INTRODUCTION

Milk and dairy fat products play a significant role in a nutritional and balanced diet, and their consumption has been associated with various nutrition values due to their rich protein, fat, lactose, and mineral content. Butterfat, similar to milk fat, is primarily composed of mono-, diamonded, and triglycerides, phospholipids, and cholesterol. It also contains a high concentration of short-chain fatty acids, which possess biological functions and serve as a carrier for some nutrients, particularly lipid-soluble vitamins. Moreover, milk fat is a critical factor affecting the physiochemical characteristics, nutritional value, and economics of milk and milk products (Saeed et al., 2019). Adulteration , though an illegal practice, is employed by some food manufacturers and suppliers to increase profits (Deelstra et al., 2014). It poses a considerable concern for customers as it may affect their health, food quality, food safety, and loss of importance in food products (Eisenstecken et al., 2021). Dairy products are among the most adulterated food products. Specifically, milk fat is often substituted with fats or oils of other origins because of their high profitable value and price. Butter, a natural food product and one of the most expensive edible fats, costing about nine to ten times more than palm oil. The term "butter" is legally protected within the European Union, designating a product made exclusively from milk. In the production of butter, pasteurized cream, starters, and natural coloring are only admissible. However, Products containing foreign fats of plant or animal origin However, “butter“. Developing methods for monitoring the evolution of industrial processes has become of great interest. (Muhammad Nadeem et al., 2017).

Whey proteins are an excellent nutritional source known for their bioactive components. They are rich in bioactive peptides, branched-chain amino acids such as leucine, valine, and isoleucine, as well as essential amino acids such as cysteine. Due to their anti-inflammatory, antioxidant, hypotensive, anti-obesity, and appetite-suppressing properties, whey proteins possess high biological and nutritional value. Furthermore, whey proteins exhibit essential beneficial properties in food processing, including carrier agents, gelling agents, fat replacers, and emulsifiers. They are known to improve the texture and taste of food products (Yigit et al., 2023).

The Codex Alimentarius classifies spreads and blended as margarine containing more than 80% fat and spreads containing less than 80% fat, with a butter content less than 3%. Blended spreads, on the other hand, may contain more than 3% butter. Blended spreads contain less than 80% fat, while blended may contain more than 80% fat. Butter, in contrast, must be made entirely from milk or dairy products, contain 80% fat, and no more than 16% water. All of these edible fats are water-oil emulsions (Vithanage 2009). They represent a contemporary, healthy product with a balanced blend of vegetable oils and milk fat, and of a quality comparable to that found in Europe.

Therefore, the aim of our study is manufacture a spreadable butter-like product (blended butter) at a reduced cost utilizing natural butter and vegetable oil (palm oil) and supported by whey protein concentrate to increase nutritional value and improve the texture properties.

## MATERIAL AND METHODS

### MATERIALS

Natural Butter (83% fat, 16% moisture) was obtained from Fonterra Inc., New Zealand. Palm oil (melting point 37º C) was obtained from Ajwa for food industries CO. Egypt.

Permeate whey (3.07 g/100g) and Whey Protein Concentrates (WPC 83.70% protein) was obtained from Cremo SA products: *laiters, beurre, and fromage*, Switzerland.. Lecithin was obtained from Alexandria Co. for Seed Processing & Derivatives, Egypt. Butter Flavor was obtained from IFF, (International Flavors & Fragrances), Germany. The emulsifier (monoglyceride powder, Plasgaard ®DMG 0093) was obtained from Plasgaard Denmark.

The Beta Carotene color, 31.1% was obtained from AWA for Food Additives Company. The antioxidant (tert-butylhydroquinone, T.B.H.Q.) was obtained from Arabia Co. origin India. The preservative (potassium sorbate) was obtained from Jiandbel Additives Co. LTD. Development, Rugaoport, Rugao, Jiangsu, China.

### METHODS

**Preparation of Blended Butter**

Five batches of blended butter supplemented with different concentrations of whey protein were prepared. Table 1 shows the ingredients and treatments; the control was made without added WPC, and different mixtures of blended butter with varying concentrations of WPC were manufactured at SAKR Group Company for food products Borg Al-Arab, Alexandria, Egypt. Three replicates of each treatment were carried out.

**Analysis of blended butter**

pH value was measured by using a pH meter (Crison instrument, Spain). Titratable acidity as lactic acid, free fatty acids and peroxide value were determined according to AOAC (2016).

The active substances of thio-barbituric acid (TBA) were determined using the method described by Dieffenbacher and Pocklington (1992).

The melting point was determined according to the methods described in AOAC (2026). Trace elements were measured by atomic absorption spectrophotometer according to Lajunen (1992).

**Fatty acid profile analysis**

Fats from the blends were extracted, and fatty acid methyl esters were prepared according to El-Nabawy et al., (2023). The fatty acid profile was analyzed using gas chromatography (GC), specifically an Agilent nstrument equipped with a Tg-220MS column. Nitrogen gas ($N_2$) was used as the carrier gas at a flow rate of 2 ml/min. The flame ionization detector (FID) temperature was set at 215°C. Split injection (5:1) was performed at 215°C, and the oven temperature program was ramped from 140-215°C at a rate of 4°C/min.

**Texture profile analysis (TPA)**

The TPA was performed out using a Brookfield CT-3 texture analyzer (Brookfield Engineering Laboratories, USA). Samples were cut into cubes of 3 cm³ at 10°C and compressed to 50% using probe TA15. The texture parameters (hardness, adhesiveness, springiness, and resilience) were calculated according to the International Dairy Federation (1990) and Awad (2011).

**Sensory Evaluation**

The sensory evaluation was conducted by trained panelists , including university staff, graduate students, and dairy company engineers. The sensory evaluation form included the following attributes: color (15 points ), flavor (40 points), texture (30 points), oiliness (15 points), and an overall acceptability (100).

**Microbiological tests**

These microbiological tests are crucial for determining the shelf life and potential health risks associated with the product, providing valuable information for both producers and consumers.

Nutrient agar was used for the enumeration and count of total aerobic microorganisms (Difco MANUAL, 2009); Violet Red Bile Lactose Agar was used for the count of the coliform bacteria (Bridson, 2006), while Dichloran Rose-Bengal Chloramphenicol Agar was used for enumeration and count the yeast and mold (Bridson, 2006).

**Storage and sampling**

Blended butter samples were stored in the refrigerator at 5°C and freezer at -18°C for 6 months. Samples were taken at 0, 3, and 6 months for chemical,microbiological,and texture profile analysis, and sensory evaluation.

**Statistical analysis**

Analyses of variance (ANOVA) were performed to determine treatment differences, following the methods outlined by Steel and Torrie (1980). Statistical analyses were conducted using ANOVA as an F-test and Least Significant Difference (L.S.D.) procedures within the SAS software package (version 9.13 2007). The experimental design was a split plot with three replications. The main represented three storage durations (0, 3, and 6 months). The subplots were assigned to four treatments.

**RESULT AND DISCUSSION**

Physiochemical analysis of palm oil:

Table (2) presents the physiochemical analysis of palm oil, conducted to assess its freshness and suitability as a food ingredients. The pH values and titratable acidity of the palm oil 5.82 and 0.26% respectively. Free fatty acids were 0.073% for palm oil. Cornelius **(1977)** reported free fatty acids levels (as palmitic %) of 3 for crude palm oil and 0.05% for refined palm oil.. The peroxide value (PV) and the thiobarbituric acid (TBA) alue of the palm oil were 0.2 and 0.28 meq $O_{2/kg}$ of oil, respectively.. **Cornelius (1977) also reported a PV** of 0.4 meq $O_2$/kg oil for the refined palm oil. The melting point of palm oil (37°C); which aligns with the range of 30.8-37.6 °C reported by Fereidoon Shahidi (2005).

**Composition of fatty acids**

The fatty acid composition of palm oil, determined by gas chromatography (GC) analysis, is presented in Table 4. . Medium-chain saturated fatty acids (C12-C18) were 60.12%, while unsaturated fatty acids (C18:1-C18:2-C18:3) were 39.02%. Notably, Palm oil exhibited a high percentage of saturated fatty acids, with palmitic acid (C16:0), comprising 57.13%. These findings are consistent with those reported by Frank D. Gunstone (2002), who observed palmitic acid (C16:0) levels of 57.3% in Brazilian palm oil, but lower in Malaysian palm oil (40-47.8%). The palm oil used in this study is shortening as a palm oil fraction not hydrogenated, and the melting point is 36-38 °C. Therefore, the fatty acid profile accurately reflects the characteristics of the material used. .

**Analysis of manufactured blended butter**

Physiochemical analysis of blended butter:

The physicochemical properties of blended butter were analyzed, focusing on the effects of whey protein concentrate (WPC) substitution and storage conditions. Table2 presents the change in pH and titratable acidity of butter blended with 25% (B1), 50% (B2), 75% (B3), and 100% (B4) WPC, respectively, over a 6-month storage period at both refrigerated (5°C) and frozen (-18°C) temperatures. The pH values of control and replaced with whey protein concentrate (WPC) samples (fresh and stored) ranged from 4.1 to 5.9 . This range is consistent with the findings of Simsek (2011), who reported that the pH of fresh and stored butter samples varied between 4.38 ± 0.11and5.25 ± 0.08 , However, the pH values observed in this study were slightly higher than those reported by Atamer et al. (2004), which ranged from 3.63 to 4.71. Also, the results showed that the pH values were a significant difference between control and samples (LSD 0.063 at *P≤0.05*). During storage, a significant decrease in pH was observed for all treatments, Furthermore, storage temperature significantly influenced pH, with samples stored at 5°C exhibiting lower pH values than those stored at -18°C. Similarly, titratable acidity showed a significant increase (LSD = 0.174, P ≤ 0.05) with increasing WPC substitution, reaching 1.01% for B4 compared to 0.91% for the control. After 6 months, titratable acidity was higher in samples stored at 5°C than in those stored at -18°C. Similarly, titratable acidity showed a significant increase (LSD = 0.174, P ≤ 0.05) with increasing WPC substitution, reaching 1.01% for B4 compared to 0.91% for the control. After 6 months, titratable acidity was higher in samples stored at 5°C than in those stored at -18°C. In control, B1, B2, B3, and B4 samples titratable acidity was determined as (1.12 and 0.92%), (1.12 and 0.95%), (1.14 and 0.98%), (1.23 and 0.98%), and (1.27 and 1.02%) at 5°C and -18°C, respectively. There were significant differences (*LSD 0.174 at P≤0.05*) between storage periods. These values were higher than values found by Atamer ***et al.*** **(2004),** who recorded titratable values of cream and yayik butter as 2.36°SH (0.0531%) lactic acid and 4.19°SH (0.094% lactic acid) at the end of the storage period.

**The melting point of blended butter:**

Table 2 indicates no differences in melting points between the control and replacement samples. The melting points of all samples were measured at zero time and after six months of storage, yielding values of 38°C and 37°C, respectively. This decrease showed no statistical significance (LSD = 1.258, p ≤ 0.05). Further analysis is needed to explain the slight decrease in melting point during storage, and to clarify the composition of the control and replacement samples.

**Stability of blended butter (Free fatty acids (FFAs), peroxide values (PV), and Thiobarbituric acid (TBA)**

Table 3 shows that replacing of whey permeate with whey protein concentrate resulted in slight changes in free fatty acid (FFA) values, which were 0.257% and 0.285% for the control and B4 samples, respectively. FFA values were higher in butter samples stored at 5°C (refrigerator) compared to those stored at -18°C (freezer). After 6 months of storage, the maximum values were 0.316 % and 0.358% for control and replaced B4.

There were no statistically significant differences between treatments, nor were there significant differences related to temperature or storage period (LSD = 0.049, $P \leq 0.05$). . Studies have reported maximum FFAs values in cream butter as 1.53 mg KOH/g and 1.36 mg KOH/g (Atamer, 1983)s. Senel (2006) observed FFAs values as 1.07mg KOH/g in yayik butter. Atamer et al**.** (2004**)** found that the FFAs ranged from 0.72 to 13.92 mg KOH/g in yayik butter collected from the market samples. The FFA values obtained in the present study for all samples were within the range of these previously reported values.

Table 3 illustrates the changes in peroxide values (PV) of blended butter during storage. Initially, There were no significant differences in PV between control and replacement samples. Subsequently, (PV) increased during 6 months of storage from 0.2 at zero time to 0.9 and 0.4 after 6 months at 5°C and -18°C, respectively. Ozkan et al. (2007) reported that PV changed from 0.35 to 1.00 and 1.14 meq/g at 5°C and 20°C, respectively. The effect of storage temperature and storage period was found to be significant (LSD 0.014 at $p \leq 0.05$).

Table 3 shows that replacing up to 100% of whey protein concentrate (WPC) did not significantly affect thiobarbituric acid (TBA) values, which remained at 0.048 nmol malondialdehyde/kg across all samples. Statistically, there were no significant differences between the treatments.

However, a significant increase in TBA values was observed during storage at 5°C (LSD = 0.113 $P \leq 0.05$), while the increase at -18°C was not significant. As expected, TBA values were higher in samples stored at 5°C than those stored at -18°C. Ozturk and Cakmaki (2006) indicated that the TBA values of the butter samples increased during storage at 5°C. Simsek (2011) observed that the highest TBA values (0.37 ± 0.02 nmol malonaldehyde/kg) were in butter stored at 20°C.

**The trace elements (zinc, cadmium, and lead) of blended butter:**

Non-significant differences were observed in zinc concentration between control and replacement samples, nor between the different storage temperatures (LSD 0.037 at $p \leq 0.05$) were regarded. These results were much less than 5.98±0.407 ppm, which was recorded by Ankur et al. (2022**).** Cadmium and lead were notdetected in any of the samples.

**Contents of determined fatty acids of blended butter by gas chromatography (GC)**

Table 4 presents the fatty acid profile of blended butter samples (C, B1, B2, B3, and B4) as determined by gas chromatography (GC) analysis. . Nine fatty acids were identified in the samples (lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and, behenic acid). Palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) were the major fatty acids, comprising 93% of the total identified fatty acids. The saturated fatty acid content was higher than the unsaturated fatty acid content in both the replacement and control samples, ranging from 51.59% to 52.32% and 45.60% to 45.34%, respectively. Although the ratio of them was about 0.88-0.86, which was lower than for the American Medical Association (LO and Handel, 1983**)** of a minimum ratio of 1.2, the replacement samples were more stable than control during storage for 6 months. During storage periods for 6 months, total saturated fatty acids significantly increased in samples stored at 5°C (LSD 0.023 $p \leq 0.051$).

Total unsaturated fatty acids content significantly decreased in all samples stored at both 5°C and -18°C, probably due to the low content of polyunsaturated fatty acids and adding WPC. (**Nadeem *et al.*, 2016)** reported that the fatty composition of blends of butter oil and mango kernel oil in ambient and accelerated oxidation (25 and 55°C) was different from fresh samples.

**Texture analysis of blended butter**:

The rheological properties of blended butter, where whey permeate was replaced with whey protein concentrate (WPC 80% protein), were examined . The control sample contained no added WPC. Consistency, a key rheological property, is defined as a material's resistance to permanent deformation. . For butter, several factors are of importance of separability of final product. Butter should be firm against sagging under its own weight; sagging may also go along with oiling off, which is quite undesirable. Butter should be readily spreadable, without being too soft or crumbly. It should be easily deformed in the mouth, without feeling greasy; the latter implies that the fat should melt completely at 35°C. Texture has been defined as the external manifestation of structure and composition. In the present study, hardness, adhesiveness, resilience, and springiness were determined for all samples stored at 5°C and -18°C for 6 months; the obtained results have been presented in Figures 1: 4.

The hardness index, defined as the weight of the cone assembly divided by the penetration depth (DeMan et al., 1991), significantly increased with higher whey protein concentrate (WPC) replacement ratios. The results showed a significant gradual increase in hardness with increasing the replacement ratio of WPC; B4 recorded the highest hardness; the values were 2229 and 7179 g of control and B4, respectively. Also, the data have shown the effect of temperatures and storage periods, the hardness significantly with the increasing of the storage period, and the values of hardness of samples stored at -18°C were higher than samples stored at

5°C. The hardness significantly increased (LSD 1.166 at p≤0.05) with the increase in the storage period. The control and all samples followed the same trend, where the hardness values increased from (2229, 4340, 5300, 6348, and 7179 g) at zero time to (2561 and 2984 g), (4696 and 4784 g), (7370 and 7970 g), (8100 and 9987 g), and (9348 and 10562 g) after 6 months for control, B1, B2, B3, and B4 stored at (5°c and -18°c) respectively.

Adhesiveness decreased with increasing whey protein concentrate (WPC) replacement and prolonged storage, with lower values observed at -18°C compared to 5°C across all samples. Statistical analyses revealed significant differences between treatments, temperatures, and storage for all parameters. Hardness of blended butter is an attribute that directly correlates with the moisture content, fat content, and the type properties of the natural component in the blend. In our study, the blended butter was prepared with a fixed blend for control, and treatments were the ratio of replacement of whey permeate with whey protein concentrate (WPC), where its functional properties are well bonded to water.

On the contrary, the resilience and springiness significantly increased with increasing replacement rate; the values were 0.08 and 9.55 mm of resilience and springiness length for B4 at 1st day of storage, respectively. tatistical analysis showed significant differences in resilience and springiness during storage, but no significant differences between storage temperatures.

**Sensory evaluation of blended butter:**

Sensory evaluation of blended butter samples, as presented in Figure 5, ) and revealed that replacing whey permeate with whey protein concentrate (WPC) affected the sensory properties of samples parallel as the rating given their color, flavor, texture, oiliness, buttery taste, and overall acceptability increased; treatment B3 recorded the highest rating (94) and was higher than the control (88) at 1st day of storage, while generally acceptability decreased for all samples after 6 months. However, overall acceptability decreased for all samples after 6 months. Treatment B1 received the lowest total score. These findings align with Simsek (2011), who detected that sensory quality decreased when the storage process was ended. The results also showed there were significant differences (LSD = 2.036 at $p \leq 0.05$) in terms of statistical analysis of organoleptic data in all samples during storage between control and treatments and between temperatures and storage periods.

**Microbial content of blended butter samples**:

No viable microorganisms were detected in any manufacturing blended butter samples across all storage periods (0, 3, and 6 months) when cultured on plate count agar, violet red bile lactose agar, or Dichloran Rose-Bengal chloramphenicol agar media. Meganou and Niamke (2013) and Mégnanou and Diopoh (2015) published similar data as the butter samples were free from the microorganisms, while Karagözlü and Ergönül (2008) found that total coliform and total fecal coliform counts of the Turkish butter samples were found to be between <3 and >1400 cfu/g$^{-1}$; 62% of the samples were found highly contaminated with fecal coliform microorganisms. The same results were detected by Özalp et al. (1978), who indicated that the average content of coliform microorganisms in butter samples was 5.8 cfu/g.

## CONCLUSION

This study demonstrates the successful creation of a nutritionally enhanced, spreadable butter alternative by incorporating whey protein concentrate (WPC) into blended butter formulations. Our findings reveal that WPC significantly modulated the physicochemical, functional, sensory, and rheological properties, leading to a product with improved characteristics. . All treatments had a considerable drop in pH values over the storage periods. The temperatures during the storage time varied significantly. The ideal temperature for storage is -18°C. However, samples kept in the refrigerator (5°C) had a higher acidity than samples kept in the freezer (-18°C). The replacement of whey permeate with WPC has not significantly affected the melting point. Adding WPC caused a decrease in the total unsaturated fatty acids in all blended butter samples that were made and kept at 5°C and -18°C. The highest rating (94), higher than the control (88), was achieved by blended butter samples that replaced 75% of whey permeate with the whey protein concentrate. All samples had high-quality microbiological content during the storage period at both storage temperatures. Production of a low-fat butter substitute that is aesthetically pleasing to consumers and has a good chemical composition, good rheological qualities, good sensory recipes, and healthful value by adding palm oil (68%) and whey protein concentrate. This product offers a promising avenue for consumers seeking healthier dairy options without compromising taste or texture.

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## الملخص العربي

# دراسة مقارنة الصفات الكيموطبيعية والوظيفية والحسية للزبد الممزوجة المبتكرة والمحسنة باضافة بروتينات الشرش

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الهدف من هذه الدراسة هو تصنيع زبد مستبدل قابل للفرد بزيت نخيل 68% و12% زبد طبيعي، و نسب استبدال بين راشح الشرش المجفف وبروتينات الشرش المركز، واجراء التقييم خلال التخزين علي5 م وعلي –18 م وتم تقدير الخواص الكيمو طبيعية والحسية والوظيفية والريولوجية لشبيه الزبد الناتج وزيادة القيمة الغذائية والصحية لاحتوائه علي أحماض دهنية غير مشبعة ومركزات بروتينات الشرش التي لها صفات وظيفية وعلاجية وزيادة فرص التسويق والتغلب علي مشاكل تفاوت وأرتفاع أسعار الزبد الطبيعي المصنوع من دهن اللبن . وأوضحت نتائج التحليل للخلطة البديلة للزبد أنخفاض رقم الحموضة لكل العينات المخزنة علي 5 م عند مقارنتها بنظيرتها المخزنة علي –18م . وأن هناك تغير في محتوي المعاملات لبديل الزبد المستبدلة بنسب مختلفة من الأحماض الدهنية الحرة. تراوحت نقطة التجمد لبديل الزبد بنسب استبدال مختلفة بين 37– 38 م سواء لبديل الزبد الطازج بعد التصنيع مباشرا أو بعد التخزين بالتجميد علي –18 م لمدة 6 أشهر. ووجدت زيادة في النسبة الكلية للأحماض الدهنية المشبعة مقارنة بالأحماض الدهنية الغير مشبعة وتراوحت نسبتها مابين ( 51.5– 52.32 ) % و ( 45.00– 45.34 ) %علي الترتيب . خلطة شبيه الزبد الناتج والمخزن بالتجميد علي –18م أعلي صلابة مقارنة بشبيه الزبد المخزن بالتبريد علي 5 م . سجل مخلوط بديل الزبد المحتوي علي اضافة بروتينات شرش مجففة بنسب استبدال 75% من راشح الشرش المجفف علي اعلي تقدير ونسبة تقبل بينما قلت نسبة التقبل لكل المعاملات بعد التخزين لمدة 6 أشهر. وفي النهاية كانت الخلطات المستبدلة جميعها خالية تماما من الأعداد الميكروبية علي جميع فترات ودرجات حرارة التخزين المختلفة، وذلك بسبب الشروط الصحية ونظافة المواد الخام وظروف الصناعة.