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# EFFECT OF WHEY PROTEIN ISOLATES AS A FAT SUBSTITUTION ON THE PROXIMATE ANALYSIS AND QUALITY CHARACTERISTICS OF COOKIES

# تأثر بروتين مصل اللبن المعزول كبديل للدهن على الخصائص الكيميائية والجودة للكوكيز

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## الملخّص

يُصنّف الكوكيز بأنه من البسكويت ذي العجين الطري. تتعدد الخصائص الملمسية للكوكيز مثل اللون، والطراوة، والنكهة، ولكن أبرز هذه الخصائص هو الطراوة الناتجة عن المحتوى العالي من الدهون (Zoulias et al., 2000؛ Obeidat et al., 2018). هدفت هذه الدراسة إلى توضيح آثار استبدال الدهون بـ WPIs (بروتينات مصل الحليب) بتركيزات متفاوتة (0 %، 10 %، 20 %، 30 %، و40 %) على الكوكيز، من حيث التحليل التقريبي والخصائص الحسية. لوحظ أعلى محتوى دهني في الكوكيز عند 40 % من الدهون. أظهرت البيانات زيادة ملحوظة في محتوى الرطوبة مع ارتفاع نسبة WPIs. كما قلّ محتوى الدهون في الكوكيز بعملية استبدال الدهون بالـ WPI بنسبة 1:1، وكان ذلك متناسبًا طرديًا مع مستوى الاستبدال. كما سجّلت العينات المحتوية على WPI ارتفاعًا في نسبة البروتين، أيضًا بما يتناسب طرديًا مع كمية WPIs المضافة. أما لون الكوكيز فبقي ثابتًا دون اختلاف يُذكر في كافة المستويات. ولم يؤثر استبدال الدهون بـ WPI أيّ تأثير كبير على النعومة أو مقبولية البسكويت عمومًا حتى مستوى 20 %. علاوة على ذلك، لم يطرأ أي تغيير ملموس على نكهة البسكويت عند أيّ من مستويات إضافة WPI.

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## ABSTRACT

Cookies are classified as biscuits, "soft dough". There are many textural properties in cookies, such as color, tenderness, and flavor, but the most pronounced textural property is tenderness provided by the high-fat content (Zoulias et al,2000; Obeidat et al,2018). This study explained the effects of fat replacement with WPIs at different concentrations (0, 10, 20, 30, and 50%) on cookies regarding proximate analysis and sensory characteristics. The highest fat percentage was observed in Cookies (50%). The data revealed a significant increase in moisture content with increasing WPI in cookies. Moreover, the fat replacement WPI (1:1) reduced the fat content of cookies and was directly proportional to the level of fat replacement. The samples subjected to WPI presented increased protein contents, which were directly proportional to the level of WPI added. There was no significant effect on cookie color at all WPI levels. Fat replacement with WPI had no significant effect on the softness or overall acceptability of cookies up to 20. In addition, WPI, as a fat replacer, had no significant effect on the flavor of cookies at any added level.

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# EFFECT OF WHEY PROTEIN ISOLATES AS A FAT SUBSTITUTE ON THE PROXIMATE ANALYSIS AND QUALITY CHARACTERISTICS OF COOKIES

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## Abstract

Cookies have become a universally beloved snack around the world, with their popularity transcending age groups—from children delighted by playful flavors to adults who savor them alongside coffee or tea. The global cookies market continues to expand significantly. Cookies are classified as biscuits, "soft dough". There are many sensory properties in cookies, such as color, tenderness, and flavor, but the most pronounced textural property is tenderness provided by the high-fat content. This study explained the effects of fat replacement with whey protein isolates(WPIs) at different concentrations (0, 10, 20, 30, and 50%) on cookies regarding proximate analysis and sensory characteristics. The highest fat percentage was observed in Cookies (50%). The data revealed a significant increase in moisture content with increasing WPI in cookies. Moreover, the fat replacement whey protein isolate (WPI) (1:1) reduced the fat content of cookies and was directly proportional to the level of fat replacement. The samples treated with WPI presented increased protein contents, which were directly proportional to the level of WPI added. There was no significant effect on cookie color at all WPI levels. Fat replacement with whey protein isolates (WPIs)had no significant effect on the softness or overall acceptability of cookies up to 20. In addition, whey protein isolates(WPIs), as a fat replacer, had no significant effect on the flavor of cookies at any added level. This study will help in a better understanding of the effects and benefits of replacing fat with healthier substitutions such as whey protein isolates(WPIs).

**Keywords:** Whey protein, Proximate analysis, Cookies quality.

## Introduction

Cookies are classified as biscuits with "soft dough". There are many sensory properties in cookies, such as color, tenderness, and flavor, but the most pronounced textural property is tenderness provided by the high-fat content (Hidayat and Munarko.,2024). This product is mostly prepared with flour as base ingredients 100%; margarine 42.5%; granulated sugar 25%; dry whole eggs, 5.28%; water 4.9%3; vanilla flavoring 1.00%; and salt 1.25%(Peter Ikechukwu et al.,2017). Production and evaluation of cookies from whole wheat and date palm fruit pulp as sugar substitute. *International Journal of Advancement in Engineering Technology, Management and Applied Science*, *4*(4), 1-31.. The fat content of its products aids in performing texture, mouth feel overall smoothness, a tenderized product, a good taste, and a shorter dough (Ognean, et al., 2006. The interest in the correlation between food and health has increased in health and food processing fields (Alonso et al., 2005).

Fat,is the most food component with a relationship with chronic diseases(Beunza et al., 2010). All efforts should be made to decrease the fat content of cookieswithout affecting their sensory and quality properties (Seher and Sedat, 2017). Fat substitutes are defined as large molecules that are likely

triglyceride (TG) by physical and chemical properties and are added to replace fat with gram-to-gram or one-to-one bases, which are also stable at cooking and frying, whereas fat mimetics imitate triglyceride in characteristics such as organoleptic and physical characteristics but do not add as fat substitutes as a gram to gram or one-to-one bases, such as protein- or carbohydrate-based fat replacers, and the caloric value ranges from 0--4 kcal/g and is not stable at frying because it adsorbs water and/or denatures in caramelization (Nourmohammadi et al., 2023).Whey protein isolate (WPI) is a high protein content produced by physical separation and concentration techniques from raw cow milk to isolate the minor basic whey protein lactoferrin, and lactoperoxidase which has 90% protein or more, 6% moisture, 3.5% ash, 4.5% fat and 3% lactose with varying characteristics and can enter many food products (Garba and Kaur, 2014; Prabhakaran et al.,2024.). WPIs are utilized as fat replacers in many food products, such as infant formula, weight loss products, and food, as well as for medical purposes to provide many functions, such as good emulsifying, fat-binding, gelling, whipping, and water-binding or thickening properties (Li & Zhu, 2024). When some ingredients are used in a product due to cost, allergies can be replaced with WPIs, such as eggs, and can result in the same properties, such as aeration in baked products, such as muffins, cakes, and whips into mousses and toppings (Mahoni et al.,2024 ). The WPIs produced by these two methods include microfiltration and ion exchange, which produce products with different nonprotein contents, as ion exchange provides a relatively high level of β-lactoglobulin and without glycomacropeptide (Mahoni et al.,2024 )this study aims to investigate the effects of replacing fat with whey protein isolates (WPIs) at various concentrations (0%, 10%, 20%, 30%, and 50%) in cookie formulations. Specifically, it evaluates how fat substitution influences the proximate composition—such as moisture, protein, fat, ash, and carbohydrate content—as well as key sensory attributes, including color, tenderness, and flavor. The significance of this research lies in its potential to support the development of healthier baked products by reducing fat content without compromising sensory quality.

## 2.Materials and Methods

### *2.1. Materials*

To produce cookies, the following components were used in g: flour 100, margarine 42.5, Granulated sugar 25, dry whole eggs 5.28, water 4.9, vanilla flavoring 1.00, and salt 1.25(PeterIkechukwu et al.,2017).

### *2.2. Methods*

The preparation of cookies is shown in figure 1 :

**PREPARATION OF COOKIES**

(based on Zoulias et al. (2002)

| Step | Procedure |
|---|---|
| 1 | Margarine, sugar, salt, and dry whole eggs were mixed in a mixer at low speed for 30 seconds, then at medium speed for 5 minu- |
| 2 | Add fat substitute with water at a 1:3 ratio, at different percentages (10, 20, 30, and 50%) as shown in the table. |
| 3 | Mix the liquid ingredients with cream mixture at medium speed for 2 min. |
| 4 | Add the required amount of sugar. |
| 5 | Add flour and sodium bicarbonate to the mixture; mix at low speed for 15 seconds, then at medium speed for 15 min. |
| 6 | Form the dough into biscuits. |
| 7 | Bake for 20 minutes at 190 °C in an air–circulating oven |
| 8 | After two hours of 2 hours at room temperature, place the samples in PVC bags. |

***2.4.chemical composition:*** The analysis of Protein, fat, ash, moisture, and carbohydrate was measured using approximate analysis based on AACC methods (AACC 2011).

### *2.5. Sensory evaluation:*

Forty people participated in the consumer testing evaluation, which was conducted by bakery staff in Alkanater bakeris in Irbid. The test goals were communicated to the participants clearly.

Every panelist assessed every sample in the randomized complete block design that was employed. In each round, each participants assessed six distinct samples. Participantss rate how much they like cookies. The question on the 9-point verbal hedonic scale, which was labeled "extremely dislike " to " extremely like," asked participants to rate their general level of liking for the product's softness, flavor, and color.

### *2.6. Statistical analysis*

All measurements were performed either in triplicates and mean values were reported using analysis of variance (ANOVA) to determine any significant differences among treatments parameters. A t-test at 95% confidence level and the difference between mean value between group were compared by Duncan's new multiple range test analyzed using SPSS program version 21.0 (Chysirichote et al., 2011).

## 3.Results

### 3.1. Effect of fat substitution with WPIs on the chemical analysis of cookies:

As shown in Table 1, replacing fat with whey protein isolate (fat replacer) with ratios of 10, 20, 30, and 50% in cookies instead of margarine resulted in a decrease in fat content in cookies by 9.6%, 12%, 15.8%, and 30.6%, respectively. This trend is clearly reflected in Table 1, where fat content decreases progressively from 25.8% in the control cookies to 17.9% in cookies containing 50% WPI, demonstrating a strong fat-reducing effect of WPIs. Similarly, the energy content of the cookies decreased gradually with increasing WPI levels, showing reductions of 3.4%, 5.3%, 7.9%, and 11.2% for 10%, 20%, 30%, and 50% WPI, respectively. Table 1 highlights that the energy content decreased from 490 kcal in the control to 436 kcal in the 50% WPI cookies, indicating that partial replacement of fat with WPIs can effectively lower the caloric value of cookies. Table 1 also shows that the moisture content in cookies remains comparable to that of the control, with values increasing slightly from 10.3% in the control to 12.9% in cookies with 50% WPI. In addition, Table 1 indicates that protein content increased with higher levels of WPIs, rising from 5.9% in the control to 13% in the 50% WPI cookies, which reflects the contribution of WPIs as a protein source. Ash content also showed a slight increase, while carbohydrate content remained relatively stable.

**Table 1: Effects of whey protein isolates as fat substitution ( WPI on proximate analysis**

| Sample WPI | Ash% | Moisture% | Fat% | Protein% | CHO% | Energy (KCAL) |
|---|---|---|---|---|---|---|
| **cookies** | | | | | | |
| **0** | 0.78 ± 0.00 | 10.3 ± 0.32 | 25.8 ± 0.5 | 5.9 ± 0.09 | 56±0.38 | 490 |
| **10** | 0.97 ± 0.05 | 11.0 ± 0.21 | 23.3 ± 0.6 | 7.4 ± 0.29 | 56.7 ± 0.75 | 473 |
| **20** | 0.95 ± 0.04 | 11.8±0.12 | 22.7± 0.39 | 9.0 ± 0.29 | 55.8± 1.12 | 464 |
| **30** | 0.93 ± 0.2 1 | 12.6±0.21 | 20.9 ± 0.59 | 11± 0.31 | 53.9 ± 1.12 | 451 |
| 50 | 0.89 ± 0.19 | 12.9 ± 0.3 1 | 17.9 ± 0.09 | 13 ± 0.35 | 54.9 ± 1.12 | 436 |

*The values are the means of two replicates ± standard deviations.

### 3.2. Effects of fat substitution with WPIs at different concentrations on the sensory properties of cookies

The sensory assessment of cookies in this investigation was carried out through consumer testing utilizing a composite score system. According to earlier research, food composites can be modified based on how similar they are to the control and how their sensory qualities are. One of the key elements that draws participants to a product or makes them reject it is its color (Van and Stieger, 2020). As a result, the participants choose the color of each treatment for these products, and Table 2 explains the mean ratings. Replacing fat at all levels has a negligible effect on the color of cookies.

The softness scores of cookies are explained in Table 2. In cookies using WPIs as 20% fat replacement, there were no discernible effects on softness. Table 2 also provides cookies' overall taste and like scores. There were no significant differences in overall flavor found in any of the WPI-supplemented (control)

samples of cookies. The flavor scores of the samples treated with different levels of fat replacers were lower than those of the control samples. At every WPI level, there was also a negligible variation in the flavor characteristics.

## 4.Discussion

### 4.1. Effect of fat substitution with WPIs on the chemical analysis of cookies:

The similarity in moisture content to the control can be attributed to the high water vapor permeability of whey protein isolates (WPIs) (Kandasamy et al., 2021). This property allows WPIs to retain water effectively within the cookie matrix, helping to maintain texture and freshness. The increase in moisture with higher WPI levels is due to their strong water-binding capacity, which is clearly reflected in Table 1, where moisture content rises from 10.3% in the control to 12.9% in cookies containing 50% WPI. This indicates that WPIs not only replace fat but also enhance the water-holding ability of the cookies, potentially improving shelf life and softness.

These findings align with Sengar et al. (2022), who reported that replacing 10–50% of fat with fat replacers such as whey protein and polydextrose in biscuits reduces fat content while maintaining or improving quality and organoleptic properties. In the present study, a similar pattern was observed, as fat content decreased progressively from 25.8% in the control to 17.9% in cookies with 50% WPI, accompanied by a corresponding decrease in energy content from 490 kcal to 436 kcal, without negatively affecting moisture. This demonstrates that WPIs can effectively reduce the caloric value of baked goods while contributing positively to the overall composition. Similarly, Onsri et al. (2022) reported that incorporating whey protein concentrate in combination with inulin as fat replacers in cream puffs resulted in reduced fat content and overall energy, while improving product texture, sensory acceptance, and shelf stability. In the current study, the increase in protein content from 5.9% in the control to 13% in the 50% WPI cookies further supports the nutritional advantage of using WPIs. This increase, together with the maintained or slightly increased moisture content, suggests that WPIs enhance both the nutritional and functional properties of cookies, improving their quality without compromising consumer-acceptable sensory attributes. Overall, these results indicate that WPIs serve as effective fat replacers in cookies by reducing fat and energy content, increasing protein and moisture levels, and maintaining or improving textural and organoleptic properties, consistent with previous studies.

**Table 2: Effects of whey protein isolate on the sensory properties of Cookies**

| **Product properties** | **Percent of fat replacer ( WPI)** | | | | |
|---|---|---|---|---|---|
| | **0** | **10** | **20** | **30** | 50 |
| | **Cookies** | | | | |
| **Overall acceptance** | 7.1±1.95$^{a}$ | 7.41±1.56$^{a}$ | 7.07±1.63$^{ab}$ | 6.89±1.68$^{ab}$ | 6.12±2.01$^{b}$ |
| **Softness** | 7.3±1.50$^{a}$ | 7.18±1.42$^{a}$ | 6.53±1.54$^{a}$ | 5.21±1.53$^{b}$ | 5.78±1.82$^{b}$ |
| **Flavor** | 7.00±1.60$^{a}$ | 7.03±1.88$^{a}$ | 6.63±2.30$^{a}$ | 6.52±1.490$^{a}$ | 6.36±1.85$^{a}$ |
| **color** | 7.00±1.690$^{a}$ | 7.47±1.33$^{ab}$ | 7.18±1.57$^{ab}$ | 6.25±2.10$^{ab}$ | 6.71±1.76$^{ab}$ |

* Values within a row followed by a common letter are not significantly different

### 4.2.Effects of fat substitution with WPIs at different concentrations on the sensory properties of cookies

As was already mentioned, fat has the most palatable sensory qualities of all the ingredients in sweets and food products, and it tastes well in cookies . Therefore, any modifications that lower the fat content of cookies will be met with less acceptability. The reason for the decrease in flavor is that the flavors of the food are dissolved in either water or fat, and as many fat-soluble flavors evaporate from the product when fat is replaced with WPIs in food, the flavor of the dish is diminished (Kew et al., 2020). The softness scores of cookies showed no discernible effects on softness. This was related to the moisture-holding qualities of WPIs, which produced the same outcome without a difference (Serin and Sayar, 2017). Suresh and Hemalatha (2025) found that cookies formulated with whey protein isolate (combined with skim milk powder) maintained desirable texture attributes—such as crispness and lightness—thanks to a porous microstructure and enhanced moisture retention, without notably affecting softness (Suresh and Hemalatha , 2025).

Furthermore, water-soluble taste components are retained longer when fat is substituted with WPIs, and this explains why the alteration in the flavor was negligible at different levels of WPI. The negligible effect on color and softness, together with minimal changes in overall flavor, indicates that WPIs can be used as fat replacers in cookies without significantly compromising their sensory acceptability.

## Conclusions

This study investigated the effects of replacing fat with whey protein isolate (WPI) in cookies. The maximum fat content that was replaced in Cookies was 50 % and the moisture content significantly increased with increasing WPI in cookies. Moreover, the fat replacement with WPI (1:1) reduced the fat content of cookies and was directly proportional to the level of fat replacement. The samples treated wirh WPI presented increased protein contents, which were directly proportional to the level of WPI added. There was no significant effect on cookie color at all WPI levels. Fat replacement with WPI had no significant effect on the softness or overall acceptability of cookies up to 20 WPI. In addition, WPI as a fat replacer had no significant effect on the flavor of cookies at any added level:

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# تأثير عزل بروتين مصل اللبن كبديل للدهون على التحليل الكيميائي وخصائص الجودة للبسكويت

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**الملخص:** أصبحت البسكويت وجبة خفيفة محبوبة عالميًا، حيث تجاوزت شعبيتها الفئات العمرية المختلفة—من الأطفال الذين يفرحون بالنكهات المرحة إلى البالغين الذين يستمتعون بها مع القهوة أو الشاي. يُصنف البسكويت على أنه من فئة "العجينة الطرية". يحتوي البسكويت على العديد من الخصائص الحسية مثل اللون والطراوة والنكهة، لكن أكثر خاصية ملموسة بارزة هي الطراوة الناتجة عن محتوى الدهون العالي. هدفت هذه الدراسة الى توضيح الاثار الناتجة عن استبدال الدهون بعزل بروتين مصل الحليب عند تركيزات مختلفة (0، 10، 20، 30، و50٪) على البسكويت من حيث التحليل الكيميائي والخصائص الحسية. لوحظت أعلى نسبة دهون في البسكويت عند 50٪. وأظهرت البيانات زيادة ملحوظة في محتوى الرطوبة مع زيادة كمية عزل بروتين مصل الحليب في البسكويت. علاوة على ذلك، أدى استبدال الدهون بعزل بروتين مصل الحليب إلى تقليل محتوى الدهون في البسكويت وكان ذلك متناسبًا مباشرة مع مستوى الاستبدال. أظهرت العينات المعالجة بعزل بروتين مصل الحليب زيادة في محتوى البروتين، وكان ذلك أيضًا متناسبًا مباشرة مع كمية البروتين المضافة. لم يكن هناك تأثير كبير على لون البسكويت عند جميع مستويات البروتين المضافة. كما لم يؤثر استبدال الدهون بعزل بروتين مصل الحليب بشكل كبير على الطراوة أو القبول العام للبسكويت حتى مستوى 20٪. بالإضافة إلى ذلك، لم يكن لعزل بروتين مصل الحليب، كبديل للدهون، تأثير كبير على نكهة البسكويت عند أي مستوى مضاف.
الكلمات المفتاحية: عزلات بروتين، جودة البسكويت، التحليل الكيميائي.