Comparison of Omega-3 and Protein Content in Chickpea-Based Plant Patties Fortified with Different High Polyunsaturated Fatty Acid Vegetable Oils

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Abstract: Plant-based diets are gaining popularity due to health and environmental benefits. However, ensuring sufficient omega-3 intake from plant sources remains a challenge. This study examined the effects of vegetable oils rich in polyunsaturated fatty acids on the protein and omega-3 content in chickpea-based patties. Oils rich in omega-3, namely camelina oil (F1), canola oil (F2), and soybean oil (F3), were added based on the recommended daily intake. Protein content was measured using the Kjeldahl method. The control patty (F4, without oil) had the highest protein content (9.96%), significantly higher than F1 (9.12%), F2 (8.60%), and F3 (8.69%) ($p < 0.05$). F1 had the second-highest protein content and was significantly higher than F2 and F3 ($p < 0.05$). F2 and F3 showed the lowest protein levels and were not significantly different from each other ($p > 0.05$). Among oil-added samples, F1 showed the highest protein content. This may be attributed to camelina oil's better compatibility with the patty matrix, causing less protein dilution compared to canola and soybean oils. Although camelina seeds contain less protein than soybean, their integration into the formulation may preserve protein content more effectively. These patties offer a practical option to improve nutrient intake, especially among children.

***Keywords**: Vegan patty, Plant-based protein, Omega-3, Vegetable oils, Protein content*

1.0 INTRODUCTION

The global demand for sustainable and health concern on food alternatives has fuelled significant innovation in plant-based protein sources. Chickpea (*Cicer arietinum*) stand out as a highly versatile legume, offering a nrich nutritional profile including protein, dietary fibre, and various essential nutrients. Their inherent properties make them an excellent based developing plant patties, as alternative for traditional meat products. The allergen free properties of chickpea also make it more suitable as soybean replacer in commercial plant-based patty. However, despite chickpea being a multi-nutrient ingredient for plant-based patties, these foods often lack of nutrients essential nutrients vital for human health which is omega-3 fatty acid.

Omega-3 fatty acids, notably alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are essential nutrients vital for human health, playing crucial roles in cardiovascular function, brain development, and reducing inflammation. Given that the human body cannot synthesize these fatty acids, it is must be consumed from dietary intake such as from fish and marine source. As fish is an animal-based ingeredient, its rich omega-3 content can't be incoperated in the plant-based patty as per vegan requirement.

As a solution, omega-3 fatty acids are can be found from plant-based origins specifically from vegetable oils like camelina, canola, and soybean oils. These oils are particularly rich in ALA (alpha-linolenic acid), a type of omega 3. This makes them ideal for incoperating into plant-based patty by offering essential omega-3 to the consumers and helpig them to complete their daily nutritional intake despite within a fully plant-based food.

Other than as omega-3 source, fortifying these oils into the plant-based patty also could offer functional and sensory improvements while alligning with nutiritional trends. To provide the enough amount of omega-3 in the patty, varying oil dosage will also affect on the nutritional profile. Hence this study is aim to comparing the protein content and amount of fatty acid present in the chickpea plant-based patty that fortified with different types of vegetebale oils, which are camelina, canola and soybean oil. The findings of this research will contribute valuable insights for the food industry in formulating nutritionally superior and appealing plant-based protein products, addressing the increasing consumer demand for healthy, sustainable, and convenient food options that are rich in essential fatty acids and protein.

2.0 LITERATURE REVIEWS

Omega-3 fatty acids are indispensable for human health, playing critical roles in cardiovascular well-being, neurological development, and inflammatory modulation. As these essential fatty acids cannot be synthesized endogenously, their dietary acquisition is paramount. While marine sources, such as fish, are renowned for their high omega-3 content, the increasing demand for plant-based alternatives necessitates the identification of viable non-animal sources. Plant-based omega-3s, predominantly in the form of alpha-linolenic acid (ALA), offer a suitable alternative, as the human body can endogenously convert ALA into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Pandey A. et al., 2025). This biological conversion underscores the significance of incorporating ALA-rich plant sources into the diet.

Within the realm of plant-based omega-3s, various vegetable oils stand out due to their high polyunsaturated fatty acid (PUFA) content.[3]Camelina oil (Camelina sativa) for instance, is a particularly rich source that containing approximately 35-40% ALA (Rakita, S. et al., 2024), positioning it as one of the most potent plant-based omega-3 reservoirs. Similarly, canola (Brassica napus) and soybean oil (Glycin max) contribute appreciable amounts of ALA, providing about 11% and 9% respectively (Shahraki, M. et al., 2022). These oils collectively represent a viable and accessible alternative for individuals aiming to augment their omega-3 intake without recourse to fish or nuts, thus bridging a crucial nutritional gap for those adhering to plant-based diets.

Complementing the nutritional benefits of these oils, chickpeas serve as an excellent foundation for plant-based food products due to their robust protein profile.Chickpea flour typically contains 20–26% protein with isolates and concentrates exhibiting even higher concentrations (Miedzianka et al., 2022; Summo et al., 2023). Beyond quantitative protein content, chickpeas offer a commendable balance of essential amino acids, making them ideal for plant-based protein fortification despite a common limitation in methionine (Summo et al., 2023). Furthermore, their inherent water- and oil-holding capacities are advantageous for crafting cohesive and palatable meat alternatives with desirable juiciness (Zhang et al., 2023). These intrinsic properties of chickpeas position them as a prime candidate for developing nutritionally enhanced plant-based patties.

***Figure 1**: Chickpea*

In addition to their protein contribution, chickpeas are naturally endowed with dietary fiber and beneficial unsaturated fatty acids, including linoleic and α-linolenic acids, which further elevate the nutritional and functional appeal of formulated patties. The complex matrix formed by chickpea effectively supports protein gelation, which is instrumental in dictating the final product's texture and mouthfeel attributes critically important for consumer acceptance. This synergistic relationship between the inherent qualities of chickpeas and the potential for added fortification paves the way for comprehensive nutritional improvements in plant-based alternatives.

The strategic incorporation of vegetable oils into plant-based patties not only serves as a vital omega-3 source but also aims to improve the overall functional and sensory attributes of the product. While oils are typically added for textural enhancement and mouthfeel, their presence can also influence the protein content, primarily through dilution, especially in formulations with lower baseline protein levels. Interestingly camelina oil is unique among these choices as it not only boasts over 50% polyunsaturated fatty acids but also contains a modest amount of protein (Heuzé et al., 2020), which could mitigate some dilutive effects. Conversely, canola and soybean oils, while rich in PUFAs, have negligible protein content and may instead alter protein digestibility and retention during processing (Zhang et al., 2023). The specific fatty acid profiles and oxidative stabilities of these high-PUFA oils are also critical, as they can differentially impact emulsion stability, cooking characteristics, protein denaturation, and ultimately, the nutrient retention and textural integrity of the final patty.

Given these complex interactions, precise protein analysis in fortified plant-based patties becomes paramount for accurate nutritional labeling and consumer guidance. The addition of oils, particularly those with little or no protein, can lead to a reduction in the overall protein percentage per gram due to fat dilution. Moreover, the thermal and functional interactions occurring between proteins and lipids during patty preparation can profoundly modify protein quality and bioavailability (Miedzianka et al., 2022). Specific oils may also influence protein oxidation and denaturation rates during cooking, directly impacting both textural integrity and protein digestibility. Therefore, utilizing established quantification techniques, such as the Kjeldahl or Dumas methods, is essential to comprehensively evaluate the nutritional implications of oil fortification in meat analogs, ensuring that the enhanced omega-3 profile does not inadvertently compromise protein content or quality.

3.0 METHODOLOGY

3.1 Reagent and materials

Chickpeas, camelina oil, canola oil, soybean oil, textured vegetable protein (TVP), high-gluten flour, and seasoning were the primary ingredients used in the patty formulation. All materials were obtained from local commercial suppliers in Pagoh, Muar, Johor and were food-grade quality. Reagent and solvent used for fat extraction and protein is purchased from Sigma-Aldrich.

3.2 Production of omega-3 plant-based patty

Chickpeas were soaked overnight and steamed for two hours. Once steamed, they were coarsely minced using a KitchenAid food processor. Likewise, textured vegetable protein (TVP) was soaked in boiling water for 10 minutes and then minced with the same food processor. All ingredients were weighed according to the formulation in Table 3.2, combined, and mixed using a bowl-lift stand mixer. The patties were made with camelina oil (Formulation 1), canola oil (Formulation 2), soybean oil (Formulation 3) and and without vegetable oil (control) to compare on omega-3 and protein level.

Table 1

Formulation of plant-based patty.

Ingredient	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Chickpea	30%	30%	30%	35%
TVP	30%	30%	30%	35%
High gluten Flour	25%	20%	15%	30%
Salt and seasoning	5%	5%	5%	5%
Camelina oil	10%	-	-	-
Canola oil	-	15	-	-
Soybean oil	-		20	-

3.3 Cooking method

All plant-based patties samples F1, F2, F3, and control were cooked using the same method and condition. Both were cooked on pan using induction cooker set to pan-fry mode. Each side of the patties were cooked for 5 minutes or until fully cooked. No external oil were added on the pan during the cooking to avoid interference of omega-3 fatty acid reading.

3.4 Protein analysis

To ascertain the protein content within the sample, this study employed the Kjeldahl method, an esteemed and extensively validated analytical technique renowned for its precision and reliability in protein quantification. Celebrated as the gold standard and reference benchmark for calibrating alternative protein assays, the Kjeldahl method remains indispensable in protein analysis (Sáez-Plaza et al., 2013). The analysis focused on chick-pea-based plant patties fortified with different types of vegetable oils.

For sample preparation, approximately 1–3 grams of the chick-pea-based plant patties were meticulously weighed and homogenized to achieve compositional uniformity. The homogenized sample was subsequently transferred into a Kjeldahl flask, where it was digested with 20 mL of concentrated sulfuric acid in the presence of a catalytic agent. The digestion process continued under controlled heating until the solution turned colourless, an indication of complete organic matter decomposition.

Post-digestion, sodium hydroxide (NaOH) was introduced to the mixture, facilitating the neutralization of residual acid and liberating ammonia (NH_3) gas from the ammonium sulfate formed during digestion. The released ammonia was then captured in a boric acid solution, creating a medium suitable for quantitative analysis. Titration was carried out using standardized hydrochloric acid (HCl), with the endpoint precisely determined through the application of mixed indicators such as methyl red and bromocresol green. This meticulously orchestrated protocol ensured a highly accurate determination of the ammonia and by extension, nitrogen content, ultimately allowing for the reliable calculation of the sample's protein concentration (Sáez-Plaza et al., 2013).

3.5 Omega-3 fatty acid analysis

The patties sample were submitted to Innovation Center in Agritechnology for Advance Bioprocessing, Universiti Teknologi Malaysia (UTM) Pagoh for GC-MS Analysis. The sample were analyzed according to David et al. 2005 which for sample preparation method, 100mg sample were weighed in 20mL reaction vial and dissolved in 10mL hexane. Next, 100µL 2N potassium hydroxide was added in methanol. Vial was closed and vortexed for 30 second and centrifuged. The clear supernatent were transferred into a 2mL autosampler vial. The fatty acids were then anlyzed using column 60m x 0.25mm ID, 0.15µm DB-23 (J&W 122-2361), inlet temperature at 250ºC, injection volume 1µL, split ratio 1/50, helium as carrier gas, head pressure at 230kPa constant pressure (33cm/s at 50ºC). Oven temperature at 50ºC, 1 min, 25ºC/min to 175ºC, 4ºC/min to 230ºC, 5 min. Next detector temperature 280 ºC, and detector gases were hydrogen (40mL/min), air 450ml/min, helium make-up gas: 30mL/min.

3.6 Identification and quantification

The individual omega-3 fatty acids (primarily ALA, EPA, and DHA) were identified by comparing their retention times with those of the known standards. The area under each peak was used to quantify the fatty acids by comparing it to the calibration curve obtained from the standards. Omega-3 content was expressed as the percentage of total fatty acids in the sample which then calculated into milligram according to equation [1] below.

Equation [1]

Equation [1] for conversion of fatty acid percentage into mass (mg)

$$\text{percentage of omega} - 3 \text{ fatty acid}, \% \text{ x } \text{ weight of patty (mg)} = \text{mass of omega} - 3 \text{ fatty acid (mg)}$$

4.0 DATA ANALYSIS AND FINDINGS

4.1 Protein level

Table 2

Protein composition across formulation

F1 (Camelina oil)	F2 (Canola oil)	F3 (Soybean oil)	F4 (Control)
9.1233±.00577 [b]	8.6000± .01000 [c]	8.6867± .00577 [c]	9.9567±.00577[a]

All formulations (F1–F4) in this study showed there is a significant difference in protein content across plant-based patty formulation with different types of vegetable oil as tabulated in table 1. Among them, F4, a plant-based patty made from chickpea without any added oil**,** demonstrated the highest protein content (9.9567%)**.** This result is attributed to the higher composition and concentration of chickpea in the formulation, undiluted by added fats.

Chickpeas are known for their high protein content, typically ranging from 19.3% to 25.4% depending on the cultivar and processing method (Khazaei et al., 2020; Rani et al., 2021). Their protein is also rich in essential amino acids, notably lysine, which is often limited in cereal-based products (Iqbal et al., 2022). Thus, the significantly higher protein in F4 correlates well with literature that supports chickpea as a strong standalone protein contributor in plant-based meat alternatives.

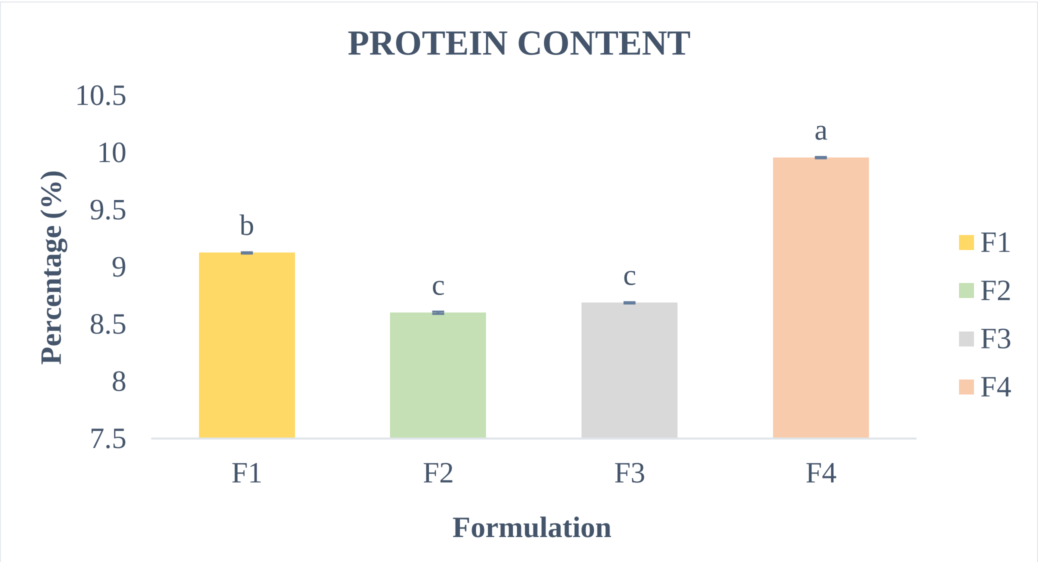

Figure 2*: Protein content (%) across formulation*

According to figure 4, the control patty (F4, without oil) had the highest protein content (9.96%), significantly higher than F1 (9.12%), F2 (8.60%), and F3 (8.69%) ($p < 0.05$). F1 had the second-highest protein content and was significantly higher than F2 and F3 ($p < 0.05$). F2 and F3 showed the lowest protein levels and were not significantly different from each other ($p > 0.05$). Among oil-added samples, F1 showed the highest protein content. Although vegetable oils do not contain protein themselves, their inclusion dilutes the overall protein percentage in the patty. Oils displace protein-rich ingredients (like chickpea) in the total formulation, reducing the relative concentration of protein per gram of sample. Additionally, certain oils may interact with proteins differently during processing and cooking, affecting extraction efficiency and structural integrity (Zhu & Zhao, 2020; Xie et al., 2021).

Among the oils used, camelina oil (F1) supported the highest protein retention among the oil-containing samples. This aligns with studies that highlight camelina oil's favorable characteristics**,** including high oxidative stability, presence of antioxidants like tocopherols, and better emulsifying behavior with plant proteins (Abramovič et al., 2020; Ghamkhar et al., 2021). These features can minimize protein denaturation during thermal processing and contribute to a more stable protein matrix.

On the other hand, canola (F2) and soybean oils (F3)**,** while widely used in food formulations, have been shown to provide less structural support to protein gels and are less effective in retaining protein content under high heat or emulsification conditions (Elmadfa & Meyer, 2021). Moreover, refined oils such as soybean and canola are essentially protein-free and contribute no nutritional protein to the formulation.

The significantly higher protein content in F4 confirms that omitting oil and maximizing chickpea content yields a more protein-dense product. However, among the oil options**,** camelina oil may be considered the most favorable in terms of minimizing protein loss**,** based on the significant difference between F1 and the lower-performing F2 and F3.

4.2 Omega-3 fatty acid level

Table 3

Omega-3 fatty acid result in before and after cooked in all patties.

Omega-3 result	Before cooked, mg	After cooked, mg
Formulation 1 (Camelina oil)	2600	1600
Formulation 2 (Canola oil)	1200	1100
Formulation 3 (Soybean oil)	1200	700
Formulation 4 (Control)	Not detected	Not detected

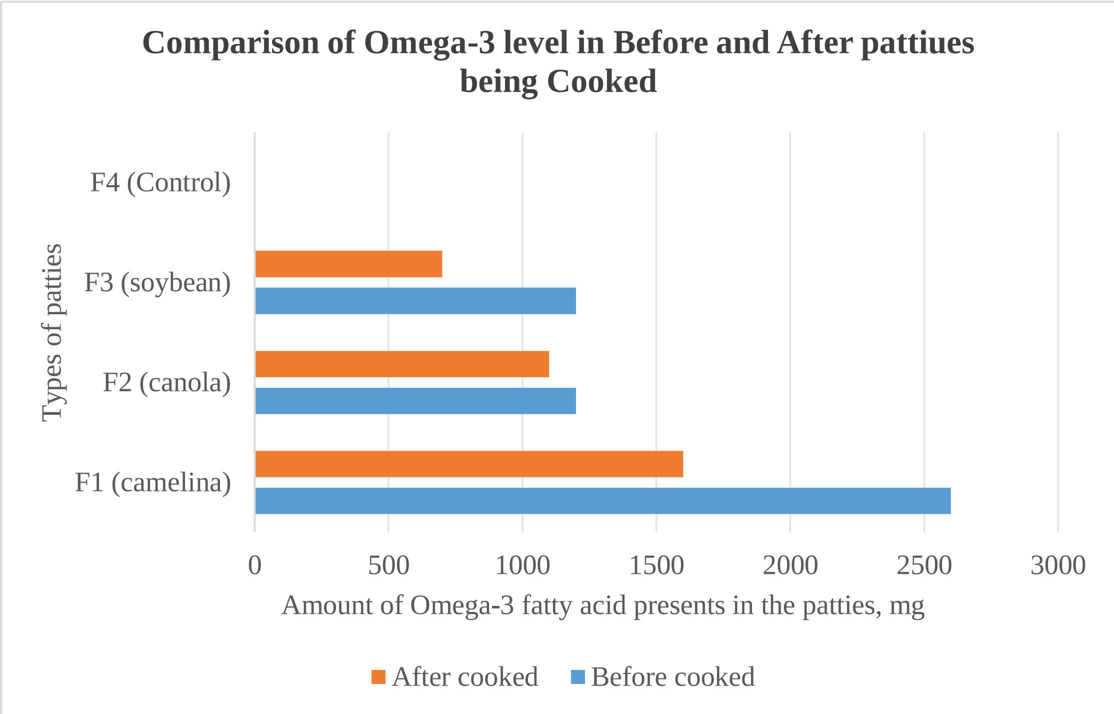

Figure 3: *Comparison of Omega-3 level in Different Formulation Before and After patties being cooked*

Table 3 and Figure 7 demonstrate the omega-3 fatty acid level in all different formulations of omega-3 plant based patties. The results show that the formulation 4 which control sample that is added with no vegetable oil indicating non detected level of omega-3 fatty acid in both before and after cooked patties. Meanwhile F1 (camelina oil) shows and increasing amount of omega-3 compared to F2 (canola oil) and F3 (soybean oil) in cooked patties even though there was 26% loss of omega-3 compared with before being cooked. F2 and F3 patties have similar level of omega-3 in the patties before cooked, but F3 illustrate more loss of omega-3 than F2. From this, it can be concluded that camelina oil which F1 could added into the plant-based patties as it can provide a sufficient amount of omega-3 level as per daily recommended dosage in range of 1200 - 1800 mg which is 1600 mg. While the others, F2 and F3 were have out of range level of omega-3 in the patties and F4 provide no omega-3.

5.0 DISCUSSION AND CONCLUSIONS

The significant differences in protein content across the plant-based patty formulations (F1–F4) are attributed to the varying inclusion of vegetable oils. The control patty (F4), without added oil, showed the highest protein content (9.96%), consistent with reports highlighting chickpea's high protein content, typically ranging from 19.3% to 25.4% depending on cultivar and processing (Khazaei et al., 2020; Rani et al., 2021). Chickpea is also rich in lysine, making it an excellent base for plant-based protein products (Iqbal et al., 2022).

Among oil-added samples, F1 (with camelina oil) retained the highest protein content (9.12%). While oils do not contain protein, camelina oil may better support protein retention due to its antioxidant content (Ghamkhar et al., 2021) and better emulsion stability, which can minimize protein denaturation during heating (Abramovič et al., 2020). Camelina oil has also shown better interaction with plant proteins in meat analog applications (Ivanović et al., 2022).

Conversely, F2 (canola) and F3 (soybean) showed the lowest protein content and were not significantly different from each other ($p > 0.05$). These oils are less effective at stabilizing protein matrices and contribute no protein to the formulation (Elmadfa & Meyer, 2021).

This study confirms that oil type significantly influences protein retention in plant-based patties. The control (F4), with the highest chickpea content, delivered the most protein. Among oil-containing samples, camelina oil (F1) supported higher protein retention than canola and soybean oils, likely due to its superior functional properties. These findings support camelina oil as a suitable choice for protein-rich vegan patty formulations.

The results indicate that incorporating various high-PUFA vegetable oils can be a promising strategy to enhance the nutritional benefits of plant-based foods, especially in terms of omega-3 fatty acid content. As shown in Table 3 and Figure 7, the control sample (F4), which contains no added meat-based ingredients, shows an absence of omega-3 fatty acids. This raises concerns about the health benefits associated with plant-based products as per also highlighted by Alam AMM Nurul et al. (2025). F1 which camelina oil shows the highest omega-3 level in both patties before and after cooked even with the lowest amount of oil with 1% of oil in it. This because camelina oil already contains high amount of ALA which can be used in food product as functional ingredient even with a small amount. Canola oil (F2) demonstrates potential in contributing to the recommended intake of omega-3s in cooked patties with 1200 mg in before and retained to 1100 mg of omega-3 after being cooked. With the lowest amount of oil loss, canola oil also seems can be the suitable omega-3 source from vegetable oil but the dosage in the formulation may be increased to meet the required levels. Similarly with F3 which uses soybean oil, even though it contains the highest amount of oil the formulation however, due to the low omega-3 content of soybean oil, the 2% inclusion is still insufficient to reach the recommended omega-3 levels thus resulting in the lowest omega-3 content among the cooked samples.

In relation with protein level, this study support the outcome that addition of high PUFA vegetable oil influenced on the protein and omega-3 level in plant-based food. Despite using chickpea as the primary protein source based for the patties, the formulations demonstrated favourable protein retention thus indicating that the addition of vegetable oil did not adversely affect the protein content. In fact, the fortification of PUFA vegetable oil in the plant based patty enhances the omega-3 content otherwise the traditional plant-based food limiting the potential health benefit to the consumer. In future, the impact of various cooking method on the omega-3 level and the texture of patty need to be studied to ensure that will not be affected from the addition of vegetable oil in the formulation.

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