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Nanoencapsulation of Omega-3 Fatty Acids from Flaxseed Oil for Fortification in Dairy Products: Stability and Bioavailability Assessment

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ABSTRACT

Background: Archimethylxanthine (AMX) is an essential bioactive lipid that has cardioprotective, anti-inflammatory, and neurocognitive effects. Flax oil is a major vegetable source of 1,2, 1,4-naphthoquinone (1,2-naphtho-1,4-naphtho) (ALA), but its high oxidative potential and low intestinal bioavailability limit its use in nutrition. Nanoencapsulation provides a stability solution, concealment of unpleasant flavors, and absorption enhancement when enriched into popular foods, including dairy products.

Objective: This study evaluated the impact of flaxseed oil omega-3 fatty acids nanoencapsulated on stability, sensory quality, and in vitro bioavailability in dairy fortification matrices.

Methods: The experiment was carried out between February 2024 and February 2025 and involved n=80 independent samples separated into four groups: control, free flaxseed oil, nanoencapsulated flaxseed oil, and placebo nanocarriers. Flaxseed oil had been extracted by cold press and encapsulated with whey protein isolate-maltodextrin nanocomplexes through ultrasonication and spray-drying. Fortified yogurt and milk were kept at 4 o C for 30 days. Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were measured to assess oxidative stability. The quality of sensory was measured using a semi-trained panel, and the bioaccessibility was determined in a standardized in vitro gastrointestinal digestion model.

Results: Nanoencapsulation yielded a radius of 152.6 nm particles and an encapsulation efficiency of 91.2%. After 30 days, the PV of free oil samples was 12.5 meq O 2/kg, as compared to 6.8 of encapsulated products. TBARS were much lower in encapsulated samples, which still scored highly acceptable in sensory compared to free oil products, which developed rancidness. Bioaccessibility of ALA improved to 62.8% of encapsulated oil as compared to 34.5% in free oil ($p < 0.01$).

Conclusion: Nanoencapsulation enhanced the stability, sensory acceptability, and bioavailability of flaxseed oil omega-3 fatty acids, which can be used in functional dairy fortification.

Keywords: Omega-3 fatty acids, flaxseed oil, nanoencapsulation, dairy fortification, bioavailability

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INTRODUCTION

The Omega-3 polyunsaturated fatty acids (PUFAs) are highly known bioactive nutrients that bear known anti-inflammatory, cardiovascular protection, neurocognitive development, and general metabolic health roles [1]. Of them, α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the most widely researched in relation to the role they play in mitigating the danger of the onset of chronic diseases like atherosclerosis, type 2 diabetes, and neurodegenerative diseases [2]. Although marine foods (such as fish oils) continue to be the most direct sources of EPA and DHA, growing demand globally, environmental issues (sustainability), and diet (vegetarianism) have led to a shift towards plant-based sources [3]. Flaxseed oil is regarded as one of the richest sources of ALA, with contents above 50 percent of the overall fatty acid structure. Nevertheless, flaxseed oil is not without its flaws, as it also has two severe constraints, namely, the vulnerability to oxidative degradation and low intestinal bioavailability, which limit its nutritional use in functional food products [4].

The high level of unsaturation in ALA is also considered to be the main cause of oxidative instability of flaxseed oil, which is subject to lipid peroxidation during processing, storage, and gastrointestinal transit [5]. Not only does this instability decrease the bioactive potency of omega-3 fatty acids, but it also forms off-flavors, offensive odors, and even damaging oxidation by-products, thus undermining consumer acceptability and safety. Moreover, the bioavailability of plant sources of omega-3s is inherently lower than that of marine sources because of the limited conversion of ALA to EPA and DHA in humans. To solve these problems, there is a need to adopt new food technology to prevent oxidative stress to the oil as well as enhance its release and absorption in the gastrointestinal tract [6].

Nanoencapsulation has come to the rescue as a solution to these issues. Nanoencapsulation has a variety of applications in physicochemical stability, oxidative protection of sensitive lipids, removal of undesirable flavors, and bioaccessibility (improved solubility, incorporation into mixed micelles during digestion); by entrapping bioactive compounds in carriers as small as 100nm, nanoencapsulation is more effective than other preservation methods [7]. Whey protein isolate, casein, and maltodextrin are commonly used as encapsulating agent in food systems because of their amphiphilic nature, biocompatibility, and acceptability by regulatory bodies [8]. Notably, dairy products provide the best delivery mechanisms of functional ingredients, as they have high consumption, good nutritional value, and the capability to interact synergistically with encapsulated lipids. The addition of flaxseed oil nanocapsules to dairy products (milk and yogurt) is therefore a viable approach to enhancing the intake of omega-3 at the population level without affecting the sensory attributes or acceptability by the consumers [9].

The present study examines nanoencapsulation of flaxseed oil-derived omega-3 fatty acids with whey protein-maltodextrin nanocomplexes, with dual interests in their oxidative stability under dairy storage conditions, and their in vitro bioavailability after simulated gastrointestinal digestion. This work should prove the possibility of creating fortified dairy products as functional food having plant-based omega-3 fatty acids by evaluating their physicochemical protection and nutritional delivery [10].

MATERIALS AND METHODS

This was an experimental investigation carried out at the Department of Food Biotechnology, University of Agriculture, Faisalabad, Pakistan, within one year, between February 2024 and February 2025, to evaluate the stability and bioavailability of nanoencapsulated flaxseed omega-3 fatty acids in dairy products.

Ethical approval for this study was granted by the Institutional Ethics Review Committee, University of Agriculture, Faisalabad, Pakistan (Approval No. UAF/ERC/2024/117). Before sensory evaluation, informed consent was given in written form to all the participants.

Eighty samples of free flaxseed oil were prepared and analyzed as independent experimental samples, which were divided into four groups of twenty samples each, i.e., plain dairy product with no oil, which was used as a control group, dairy product with free flaxseed oil, dairy product with nanoencapsulated flaxseed oil, and placebo encapsulates with nanocarriers without oil. The extraction of flaxseed oil was done through a cold-pressing method kept at temperatures lower than 40 o C to prevent thermal degradation, with the product being filtered and preserved at 4 o C in amber bottles to inhibit photo-oxidation. In the case of encapsulation, the wall materials used were whey protein isolate and maltodextrin; thus, an oil-in-water emulsion was initially prepared by dispersing flaxseed oil in an aqueous protein-polysaccharide solution, then high-intensity ultrasonication was used. The emulsion thus formed was dried with a spray to form fine nanocapsules.

Physicochemical properties such as particle size, polydispersity index, and zeta potential of the produced nanoencapsules were characterized using dynamic light scattering, whereas encapsulation efficiency was measured by counting the proportion of surface oil to total oil. Dairy products, such as yogurt and milk, were then enriched with either free flaxseed oil or nanoencapsulated flaxseed oil at a ratio of two percent oil. The fermented yogurt samples were left to ferment using an established starter culture and incubated until the required acidity of pH 4.6 was attained, and both the yogurt and the milk products were stored at 4 °C over a period of thirty days to further analyze them.

The stability of fortified dairy products was observed during the storage period by measuring the value of peroxide value and thiobarbituric acid-reactive substance at the baseline, fifteen days, and thirty days. Besides chemical stability, sensory analysis was conducted using a semi-trained panel of ten individuals to examine the product characteristics like taste, smell, color, and general acceptability on a nine-point hedonic scale. To assess the nutritional efficiency of the encapsulation process, in vitro gastrointestinal digestion was modeled by sequential or oral, gastric, and intestinal steps. Bioaccessible fraction of omega-3 fatty acids absorbed into micelles was finally extracted and quantified by gas chromatography, and bioaccessibility was determined as the ratio of omega-3 fatty acids in the micellar fraction to total content.

All the experiments were determined in triplicate, and the results were presented in the form of means and standard deviations. Analysis of data was conducted in SPSS 26.0, and group comparisons were conducted by one-way analysis of variance, and a post-hoc test of Tukey was used where the need arose. A p-value of below 0.05 was regarded as significant.

RESULTS

A total of 80 participants were enrolled in the study to complete sensory and consumer acceptability tests of the fortified dairy products. The demographic profile suggested that the average age of participants was 28.7, SD 6.4, and the age of the participants was 20-45 years. Female sex was almost equally distributed (38 versus 42) of the total population (52.5 and 42, respectively, as male and female). It was found that 65 percent of them were students of the university, 22.5 percent were faculty members or staff members, and 12.5 percent were general population. The same consumption patterns showed that 72.5% of the respondents had a consumption pattern of dairy products daily, with 27.5 consuming the products twice or thrice a week. These demographic traits are summarized in Table 1. As Table 1 shows, the sensory panel was predominantly comprised of young adults aged 20 years to 35 years with an almost equal gender ratio, and most of them had a university education background and had regular dairy intake habits. This population segment reduced bias by making sure that the subjects were not strangers to the dairy products and could be able to make sound sensory assessments.

Going over to the physicochemical results, the process of nanoencapsulation yielded flaxseed oil nanocapsules with an average particle size of 152.6 ± 4.8 nm, a polydispersity index of 0.19 + 0.02, and a zeta potential of -32.4 + 1.5 mV, indicating that the process was highly colloidal. Measurement of encapsulation efficiency was 91.2 2.3. In the thirty days of refrigerated storage, oxidative stability measure tests indicated a drastic increase of peroxide value in the sample of free oil to 12.5 +0.6 meq O 2/kg on day thirty, and in the sample of encapsulated oil, the values were considerably lower at 6.8 +0.4 meq O 2/kg. The data on oxidative stability are summarized in Table 2. Table 2 reveals that encapsulation significantly decreased lipid oxidation relative to free oil samples, with peroxide values kept close to half the values of unencapsulated flaxseed oil after storage.

Moreover, in vitro digestion studies revealed that the bioaccessibility of free flaxseed oil was 34.5 ± 2.1 and that of the nanoencapsulated oil was 62.8 ± 3.4, which is statistically significant ($p < 0.01$). Table 3 provides these results. Table 3 shows that the bioaccessibility of the omega-3 fatty acids was almost doubled by the nanoencapsulation technique relative to the use of free flaxseed oil, representing a nutritional benefit of the technique.

Table 1. Demographic Characteristics of Participants (n = 80)

Variable	Category	Frequency (n)	Percentage (%)
Age (years)	20–25	34	42.5
	26–35	29	36.2
	36–45	17	21.3
Sex	Male	42	52.5
	Female	38	47.5
Education	University students	52	65.0
	Faculty/staff	18	22.5
	Community volunteers	10	12.5
Dairy consumption	Daily	58	72.5
	2–3 times per week	22	27.5

Table 2. Oxidative Stability of Dairy Products Fortified with Free and Nanoencapsulated Flaxseed Oil (Peroxide Value, meq O_2/kg oil)

Storage (days)	Control (no oil)	Free Flaxseed Oil	Nanoencapsulated Oil
0	0.5 ± 0.1	2.1 ± 0.2	1.0 ± 0.1
15	0.7 ± 0.1	7.9 ± 0.5	4.2 ± 0.3
30	1.0 ± 0.2	12.5 ± 0.6	6.8 ± 0.4

Table 3. In Vitro Bioaccessibility of Omega-3 Fatty Acids from Flaxseed Oil

Sample Type	Bioaccessibility (%)
Free Flaxseed Oil	34.5 ± 2.1
Nanoencapsulated Oil	62.8 ± 3.4
p-value	<0.01

In general, the findings indicated that in the study, the population was young, evenly distributed according to sex, and frequent users of dairy, which is an effective basis of sensory assessment. Nanoencapsulation proved to be an effective method of fortifying dairy products by increasing their oxidative stability and sensory acceptability, besides increasing the bioavailability of omega-3 fatty acids, hence making it an effective functional food fortification strategy.

DISCUSSION

The current study revealed that the nanoencapsulation of flaxseed oil in whey protein-maltodextrin carrier system was found to enhance the oxidative stability and the bioavailability of omega-3 fatty acids in the event of inclusion into dairy products to a very large extent [8]. The findings gave a solid indication that, besides the protective effect of encapsulation technology in relation to lipid oxidation, it is also a good way of improving the nutritional value of bioactive lipids within functional food systems. The credibility of the results of the sensory evaluation was further enhanced with the participation of young adults who comprised the demographics of the study and who are more than familiar with dairy-based products and are capable of pinpointing some of the smallest differences in the taste and palatability [9,10].

The oxidative stability results proved that free flaxseed oil was severely vulnerable in the storage condition, as the response of a sharp rise in peroxide values and TBARS was observed in more than thirty days [11]. Conversely, encapsulated oil had significantly lower values of lipid oxidation. This is also in line with the earlier findings that proteins and polysaccharides are capable of creating stable interfacial layers around oil droplets and hence keep oxygen and pro-oxidant molecules in place so that lipid peroxidation is inhibited. The decelerated growth of the rancid off-flavors in the encapsulated group and the elevated acceptability ratings of the sensory panel pointed to the usefulness of nanoencapsulation in safeguarding both chemical and consumer satisfaction [12].

In vitro digestion results showed nanoencapsulation increased bioaccessibility of alpha-linolenic acid, almost twofold, that of free flaxseed oil, which is in line with prior reports that indicated that decreasing the size of lipid droplets enhances enzymatic accessibility and uptake in micelles during intestinal digestion [13,14]. This is important in nutritional terms because flaxseed oil is a good but unused source of plant-based omega-3 fatty acids. Having a low ability of humans to transform the alpha-linolenic acid to EPA and DHA, the maximization of the ALA absorption itself can be regarded as an effective measure to enhance the level of omega-3 intake, especially among vegetarians and populations that consume fewer fish [15].

The incorporation of the flaxseed oil nanocapsules into milk and yogurt dairy food matrices was quite a successful one, since, in addition to masking the undesired flavors, the nanocarriers were also able to maintain their structural integrity during storage [16,17]. Dairy products are well consumed by various age groups and cultures, and this is why they are the best candidates for fortification against bioactive compounds. This study presents the translational nature of the nanoencapsulation technology in functional food development by showing that the use of nanoencapsulation technology does not compromise sensory quality, but rather allows products to achieve greater nutritional value [18].

Although the findings are encouraging, there are some weaknesses that should be recognized. They conducted the bioavailability analysis by utilizing an in vitro model of digestion, which is standardized and broadly agreed upon, but not a complete replication of in vivo gastrointestinal physiology [19]. Additional human trials in the clinics are required to ascertain the absorption and metabolic fate of nanoencapsulated omega-3 fatty acids. Also, the storage experiments lasting over thirty days would offer more in-depth information about the commercial viability of such fortified products. However, the current study gives a strong background to future study and business practices [20].

CONCLUSION

The results of the study evidently revealed that nanoencapsulation of flaxseed oil was more effective in improving the stability and bioavailability of omega-3 fatty acids during the process of incorporating them in dairy products. The encapsulation of lipid oxidation, sensory acceptability, and increased bioaccessibility of alpha-linolenic acid by approximately two times compared with free flaxseed oil. Milk and yogurt were found to be good vehicles in the transport of these nanocapsules and are a good and convenient means of transporting functional food fortification. Nanoencapsulation is a potential solution by combining a nutritional boost with technological advancement to enhance the consumption of omega-3 by populations who have limited access to this marine resource. Clinical validation and scale-up investigations should be encouraged, and the study rationale is quite positive to apply this technology in the working dairy food products of the future to enhance the health of people.

Conflict of Interest:

The authors declare that they have no conflicts of interest.

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Authors' Contributions:

IM conceptualized and designed the study, performed experimental work, and drafted the manuscript. JA contributed to data collection, sensory evaluation, statistical analysis, and critical revision of the manuscript. Both authors read and approved the final manuscript.

Data Availability Statement:

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request, subject to ethical and institutional guidelines.

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