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ORIGINAL ARTICLE

Flaxseed mucilage - stabilized double emulsion for vitamin D delivery in Hazelnut milk ice cream: in vitro stability and storage

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Abstract

The present study aimed to fabricate a double emulsion stabilized with flaxseed mucilage containing vitamin D. The hazelnut milk ice cream was prepared with a 5 µg/100 g vitamin D double emulsion. The storage resistance and the in vitro release of vitamin D as well as the sensory characteristics were assessed. The results indicated that the highest vitamin release rate in the simulated intestinal situation for vitamin D occurs in a double emulsion structure. The observation affirmed no remarkable variations in the magnitude of vitamin D retention during 28 days of storage ($p>0.05$). The content of vitamin D in fortified hazelnut ice cream samples showed that the content of released vitamin D during in vitro simulated stomach and simulated small intestinal circumstances were about 4% and 94.8% of the initial content of vitamin D, respectively. Sensory evaluation by approach using text highlighting technique implies no remarkable variations between control and fortified hazelnut ice cream samples in various properties. Still, the purchase intention of control and enriched samples showed significant differences. After reading the highlighted text, an important difference was observed between purchase intention before and after reading the highlighted text.

Keywords Double emulsion · Vitamin D · Flaxseed mucilage · Hazelnut milk ice cream

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Introduction

Vitamins are necessary micronutrients in the diets that support the biochemical processes in the human body and ban sickness (Abbas et al. 2012). Vitamin D is a vitamin that is soluble in lipids. Vitamin D deficiency has resulted in rickets common in children, and osteoporosis in adults. In addition, extended vitamin D insufficiency is related to various non-musculoskeletal disorders, such as specific kinds of cancers, Parkinson's disease, multiple sclerosis, cardiovascular disease, diabetes type, and immune system dysfunction (Esmaeili et al. 2022). Vitamins are sensitive compounds against various factors that cause the degradation of vitamins during processing or storage. The encapsulation of vitamins can therefore solve problems related to external factors and promote efficient delivery to the body. The strategies employed for the production of functional foods should be capable of protecting the desired compounds on storage duration and after their absorption via the intestinal tract. Emulsion-based products are an alternative technique for the production of foods with functional characteristics such as the inclusion of bioactive components in the food formulation. The hydrophilic compounds could not be assembled in the continuous phase of a common emulsion because their biodisponibility diminishes after digestion (Jang et al. 2006). An alternative for the inclusion of these bioactive agents in food materials is to incorporate them into the primary emulsion of multiple emulsions, guarding them against chemical alterations in the digestion process. Double emulsions are a kind of multiple emulsion in which droplets are interspersed in the continuous phase, including other emulsions. The most popular case of double emulsion is water -in- oil-in-water emulsion ($W_1/O/W_2$), though oil-in-water-in-oil ($O_1/W/O_2$) can also be applied in specific applications (Muschiolik and Dickinson 2017). Double emulsions have lower stability rather than single emulsions, which is a major limitation of their industrial application. Coalescence, creaming, and Ostwald ripening are the major

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causative factors that led to instability in double emulsions (McClements 2010). One approach for extending the resistance of double emulsions is the application of stabilizers like hydrocolloids.

Flaxseed is also referred to as linseed (*Linum usitatissimum*(and contains about 6% mucilage. Mucilage is naturally hydrophilic, and flaxseed mucilage has two various kinds of polysaccharides: a pectic-like substance (naturally acidic) and an arabinoxylan (naturally neutral)(Puligundla and Lim 2022). Various studies focused on applying flaxseed mucilage in the fabrication of edible films (Karami et al. 2019). The application of flaxseed mucilage in foods was focused on by some researchers. Łopusiewicz et al. (2022) investigated the usage of flaxseed mucilage in fermented/non-fermented whey drinks and they reported flaxseed mucilage resulted in an increase in viscosity and consumer acceptance as well as improvement of antioxidant and nutritional value (Łopusiewicz et al. 2022. Pang et al. (2023) assessed a soy protein drink containing flaxseed mucilage and they showed that application of flaxseed mucilage caused improvement of the structure of the drink and increase in viscosity and protein particle size (Pang et al. 2023). Arabshahi- Delouee et al. (2020) evaluated the impact of flaxseed (*Linum usitatissimum*) mucilage on physicochemical and organoleptic properties of semi-fat set yogurt and they reported viscosity, consistency, water-holding capacity of the yogurt samples increased while the syneresis value decreased with enhancing the content of flaxseed mucilage and storage time. The organoleptic attributes of yogurt samples were also influenced by the amount of flaxseed mucilage and the sample containing 0.15% flaxseed mucilage was preferred in terms of all the organoleptic attributes evaluated by the panelists (Arabshahi- Delouee et al. 2020).

Text Highlighting has recently been suggested as an approach for consumer research (Jaeger & Ares, 2022). In this way, consumers are more understanding and selective because of the availability and exchange of information about the product. Knowing more deeply the parameters in relation to consumer awareness and perceptions and how information affects their decision-making has become a remarkable part of an effective strategy to direct awareness and marketing strategies in the sensory and consumer fields. So, novel methodologies have been introduced with this focus, like highlighting the text or highlighting approach. The advantage of this method is its simplicity and intuitiveness. In this method, participants should read a piece of text and mark the terms/information that they"liked" and "disliked". Text highlighting (TH) gathers qualitative data about consumers' perceptions, analyzing their attitudes toward major details about the product. In addition, TH uses a simple approach to understand the consumer's value judgment concerning information related to the analyzed food product, as in the case of the concept of a new product and the claims linked to it. Thus, TH makes it possible to understand consumers' value judgments about various kinds of data that can be applied to labels and marketing actions, such as nutritional claims. In addition, attitudes related to specific product information are part of the list of parameters in the food selection process when sensory, attitudinal, emotional, social, and stochastic parameters, among others, are present (Nogueira et al. 2023).

Since there is little research on hazelnut milk and there was no research on the fortification of hazelnut milk with vitamin D, we chose this product for our study. In this study, vitamin D was encapsulated in the internal aqueous section of a $W_1/O/W_2$ double emulsion, and the optimum condition for the preparation of the double emulsion was chosen to determine the release of vitamins in simulated gastric and intestinal circumstances. Hazelnut milk ice cream was prepared contained 5 µg/100 g of vitamin D double emulsion. Then, storage stability, and in vitro release of vitamin D and sensory characteristics were assessed. A faced-centered central composite design was exploited to distinguish the optimum magnitude of the three independent variables.

Materials and methods

Materials

Phosphatidylcholine (purity > 99%) was brought from Sigma (Germany). Flaxseed and hazelnuts were bought from a local bazaar (Neyshabur, Iran). Vitamin D was also purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemical materials were brought from Merck (Germany).

Methods

Flaxseed mucilage extraction

Flaxseed mucilage was achieved by water extraction accompanied by stirring rate was equal to180 rpm; the ratio of flaxseed to deionized water was equal to 1:8 (w/v) at ambient temperature for 18 h. Next, vacuum filtration is performed over a nylon mesh. The obtained flaxseed mucilage was freeze-dried and grounded for further use (Sungatullina et al. 2023).

Preparation of primary and double emulsion

For the preparation of double emulsion, a two-step emulsification technique was chosen according to Sawant et al. (2017), with some modifications. First, 4 mL of aqueous

phase and 0-1.5% w/v of flaxseed mucilage were blended with 6 mL of corn oil, including vitamin D and 2% w/v of lecithin and emulsified by Ultra-Turrax T-25 (IKA, India) homogenizer at 6500 rpm for 3 min. At the secondary step of emulsification, the primary W/O emulsion (10 mL) was gently mixed with 10–20 mL of distilled water, including 0.1-1% w/v of Tween 80, at 750 rpm for 10 min, applying a high-speed stirrer to produce a double emulsion (KK Sawant et al., 2017). The final concentration of vitamin D was adjusted to 10 mg/100 mL of double emulsion.

Emulsion characterization

For emulsion characterization, the following parameters were evaluated as follows:

Phase separation: The stability of emulsion samples was investigated according to the visual inspection of two separate phases: the cream layer (at the top) and the serum layer (at the bottom layer):

$$\text{Phase separation} = \frac{HL}{He} \times 100$$

Where HL and He were the height of the serum layer and final emulsion height, respectively.

Zeta potential assessment was carried out by a Zetasizer Nano ZS (Malvern Instruments Ltd., UK).

Interfacial tension was determined at room temperature by applying a Sigma 700 tensiometer (KSV Instruments Ltd., Finland) (Didar and Hesarinejad 2022).

The encapsulation efficiency of vitamin D in the double emulsion was measured by analyzing the concentration of vitamin D in the stable phase of the double emulsion by UV-visible spectrophotometry (Jenway, 6305, England). Vitamin D determination was carried out according to the method outlined by Dima & Dima. 2020. To extract vitamin D, over 10 mL mixture of isooctane and ethyl alcohol (1:3 v/v), 2 mL of sample was blended. After gentle stirring, the mixture was centrifuged (1700 g, 15 min). The concentration of vitamin D in the supernatant phase was evaluated by spectrophotometry method using a UV–VIS spectrophotometer (Jenway, 6305, England) at 265 nm wavelength. A calibration curve ($R^2 = 0.99$) was applied to calculate vitamin D content.

The particle size of double emulsion was measured by dynamic light scattering (DLS) using a Horiba SZ100 (Japan) and the dispersion index (SPAN index) is determined by the formula:

$$\text{SPAN} = \frac{\boldsymbol{d}90 - \boldsymbol{d}50}{\boldsymbol{d}10}$$

Determination of in vitro release of vitamin D in double emulsion during simulated medium

In release of vitamin D in optimal double emulsion was studied in simulated gastric fluid (SGF) under pH 1.2 and simulated intestinal fluid (SIF) under pH 6.8 by following in vitro release method outlined by Bajaj et al. (2021). Sodium chloride (2 g) and purified pepsin (3.2 g, 800 to 1200 units activity/ mg protein) were dispersed in 7 mL of 0.2 M HCl and volume made up to 1000 mL to fabricate SGF. SIF was fabricated by mixing 50 mL of 0.2 M potassium dihydrogen phosphate to 22.4 mL of 0.2 M sodiumhydroxide and volume made up to 200 mL.The sample (10 g) and their corresponding standards were taken in stoppered round bottom flasks to which the release media (SGF or SIF, 50 mL) was added and kept at 37 ºC on rotary shaker at 100 rpm. Samples were withdrawn at time 60, 120, 180, 240, 300, and 360 min (Bajaj et al., 2021).

Fourier-transform infrared spectroscopy (FTIR)

Infrared spectroscopy is one of crucial used chemical analysis approaches that gives information about molecular vibrations and provides identifies of functional groups and is, therefore, a powerful tool to identify chemical compounds, molecules and molecular segments (Marie & Torbjörn., 2007).

An FTIR spectrophotometer (Thermo, AVATAR model, USA) was applied to assess Fourier transform infrared (FTIR) spectra of double emulsion and flaxseed mucilage in the range of 500–4000 cm^{-1} at ambient temperature (Chavoshi et al. 2022).

Hazelnut milk preparation

For hazelnut milk preparation, the approach of Atalar et al. (2021) was performed with minor modifications. The first step was oil removal by cold press (HOME PRO – OPM450, Taiwan). The obtained hazelnut cake was ground by Pars Khazar (Chili Grinder, Iran) and blended with distilled water by Pars Khazar blender (Shine model, Iran) and attained the final concentration of 10% (w/v). After that, the fabricated solution was homogenized using an Ultra Turrax homogenizer (IKA-Werke GmbH & Co., KG, Staufen, Germany) (10000 rpm, 10 min) (Atalar et al. 2021).

Ice cream production

The **ice cream** production was performed as in the following formulation. 0.75 g/100 g salep as a stabilizer, 18 g/100 g sucrose, and the remainder was hazelnut milk (Atalar et al. 2021). After preheating at 65 °C and pouring all the

components, the mixture was homogenized by a laboratory homogenizer (EURO TURRAXT 20b, IKA Lobo Technik 27000 min G1), pasteurized (85 °C for 15 min) (Wang et al. 2022a) and cooled. Thereafter, it aged overnight at 5 ± 1 °C. Just before freezing in a batch freezer (Staff Ice System, BTM 10, Rimini, Italy), 0.5% vanilla was mixed into the aged ice ceam mixture. The prepared sample was placed into plastic cups, covered, and hardened in a deep freezer at -20 °C for 24 h before analysis. These steps were performed in triplicate for each batch (Abdelraouf et al. 2023).

Fortified ice cream containing a double emulsion of vitamin D was fabricated based on the method of Abd El-Raouf et al. (2023). Accordingly, all ingredients, and double emulsion were added (as the final content of vitamin D per 100 g was equal to 5 µg). Other steps were similar to the control sample described above, including preheating, homogenization, pasteurization, aging and hardening steps. The ice cream was packed into 100 mL plastic cups and stored at -22ºC (Kowalczyk et al. 2022).

Vitamin D determination in ice cream

Vitamin D determination was carried out according to the method outlined by Kazmi et al. (2007). The first step was sample preparation and pretreatment for saponification, which performed as follows: the thawed ice cream samples were diluted with distilled water at the ratio equal to 1:3. Thereafter, 1 g samples were withdrawn for vitamin D evaluation. The saponification was carried out by mixing 1 g of the diluted sample (1:3) with 0.5 ml of aqueous KOH (60%) and the tubes capped, shaken and transferred to a water bath (70ºC, 30 min). Other steps included cooling the tubes, addition of extracting solvent (methanol: chloroform), vortexing, addition chloroform, vortexing, centrifugation, separation of the chloroform phase and drying it, recosnstituent with 2 ml HPLC mobile phase and filtration. The magnitude of vitamin D was determined by high-performance liquid chromatography (HPLC) applying HPLC equipment (KNAUER, SMART, Germany) equipped with an auto injector, C_{18} column (VDSTHER) (250 × 4.6 nm). The mobile phase was methanol: acetonitrile: water (49.5:49.5:1). The volume of the injected sample was 100 µl and the flow rate was 0.3 ml/min. The elution of vitamin D was detected at 254 and 228 nm on an ultraviolet detector. The specific concentration of vitamin D reference standard was utilized for vitamin D identification and qualification in the samples (Kazmi et al. 2007) (Chansathirapanich et al. 2016).

Storage resistance of vitamin D in ice cream samples

Ice cream samples were maintained at − 22 °C throughout the research. Vitamin D retention was measured on 0, 7, 14, and 28 days of storage (Chansathirapanich et al. 2016). The approach of vitamin D measurement was according to the method of (Kazmi et al. 2007 (Chansathirapanich et al. 2016). which to be described above.

In vitro release of vitamin D in a simulated gastrointestinal condition

For the investigation of In vitro gastrointestinal digestion, the approach of Kowalczyk et al. (2022). The earliest stage was evaluation in a simulated oral stage. For this stage, a specific amount of samples (50 mL) was poured into a dark glass bottle with 100 ml volume and blended with 5 mL of simulated salivary enzyme solution fabricated by mixing and dissolving Na_2HPO_4 (2.38 g), K_2HPO_4 (0.19 g), NaCl (8 g), mucin (100 mg/L) and 150 mg/L α-amylase with enzymatic activity 200 U/L, solution per 1 L of distilled water. The blend of dissolved ice cream and saliva was regulated to pH 6.75 ± 0.20 with HCl (12 mol/L) or NaOH (1 mol/L). Thereafter, incubation in a shaking water bath at 37 °C and 90 rpm for 10 min was performed. The simulated stomach step was started by combining 13.08 mg of pepsin with the sample after the simulated oral step, and the pH was diminished to 2.0 ± 0.20 using HCl (12 mol/L). The obtained mixture was located in a shaking water bath for 2 h at 37 °C and 90 rpm. To achieve the intestinal part, the oral and gastric contents were blended with 5 mL pancreatin (4 g/L) and bile salt (25 g/L) at a pH value of 7.00 ± 0.20 (HCl 12 mol/L or NaOH 1 mol/L) and incubation proceeded for another 2 h (37 °C, 90 rpm) (Kowalczyk et al. 2022).

Determination of vitamin D after each stage of in vitro gastrointestinal stages was conducted according to the method of (Kazmi et al. 2007) (Chansathirapanich et al. 2016) which to be described above.

Organoleptic characterization of samples and acceptance experiment

The two ice cream samples were evaluated using a 9-point hedonic scale according to the method of Mohammed et al. (2020). The panelist team was ten trained panelists, six females and four males, all between the ages of 22 and 31 (Bahram Parvar et al. 2013). First, panelists (a team that included common consumers of hazelnut and dairy products) accomplished an acceptance experiment and purchase intention of samples. After that, they performed the Text Highlighting, which contained an investigation of “like” and “dislike” information about hazelnut milk, ice cream,

vitamin D, and the advantages/ disadvantages related to them. Fortified hazelnut milk ice cream acceptance and purchase intention were assessed after the text highlighting to determine the impact of information on fortified hazelnut milk ice cream acceptance (Nogueira et al. 2023).

Text highlighting

This research applied text highlighting in direct usage, centralized on recognition and variation between control and fortified samples, to evaluate the consumer's perception of samples and assess the information's influence on product acceptance. So, a text was provided to give details about the samples. This information was proposed to assess consumer awareness and perceptions of the product. Features about the advantages/disadvantages of consuming both sample groups were also included in the related text. The text possesses three paragraphs (each paragraph contemplates a main subtheme). It starts by explaining the nutritive value of hazelnut milk. Later, major information about vitamin D was given to understand the difference between the two samples. Then, some potential awareness about consuming hazelnut milk and fortified products with vitamin D was described.

A team including common consumers of hazelnut and dairy products has evaluated the different organoleptic characteristics of samples. The panel included ten panelists who like hazelnut and dairy products or are usual consumers of them. The prepared ice cream samples in cups (50 g) were served directly after withdrawing the cups from the freezer chest (storage at − 22 °C) and quickly presented to the panelists. Samples were given as random codes and presented solely in a random manner to each panelist (Abdeldaiem et al., 2023). Properties evaluated by panelists included taste, preference, appearance, and consistency via the 9-point hedonic test, where a score of 1 implies a very low desirable, and 9 implies a very high desirable (Mohammed et al. 2020). The purchase intention was evaluated by a 5-point scale, ranging from "definitely would not buy" to "definitely would buy"(Nogueira et al. 2023).

First, panelists participated in the acceptance experiment and purchased intention of hazelnut milk ice cream (control and fortified). Serving orders were regulated based on the method pointed out by Wakeling and Macfie (1995)(Wakeling and MacFie 1995). Then, the panelist completed the text highlighting acceptance and purchase intention for fortified samples.

Experimental design and statistical analysis

A faced-centered central composite design (FCCD) was chosen to specify the optimum magnitude of the three independent variables: (flaxseed mucilage content : 0-1.5%, Tween 80 content: 0.1-1%, and external water phase volume: 10–20 mL). The data was analyzed using the Design-Expert software (version 13, Stat-Ease Corporation, Minneapolis, MN, USA) by fitting the second-order polynomial model.

The statistical significance was supposed to be at $p < 0.05$. The sufficiency of the achieved models was distinguished by statistical parameters (R^2, adjusted-R^2, and coefficient of variation (CV).

All analytical examinations were accomplished in triplicate. Data were analyzed by the analysis of variance (ANOVA), and a p-value lower than 0.05 was marked as significant in surface response analysis. Three-dimensional response surface analysis was used to find the optimal level of parameters.

Analysis of Variance (ANOVA), accomplished with the least significant difference (LSD), was applied ($p < 0.05$). Analysis was accomplished via SPSS Software (version 29, SPSS Inc., Chicago) (Wong et al. 2021).

The Wilcoxon test analyzed acceptance and purchase intention data to differentiate the results obtained for control and fortified hazelnut milk ice cream samples before performing the text highlighting and to compare fortified sample acceptances pre and post-performing text highlighting. Results were depicted by boxplot, utilizing the median (Scudino et al. 2023). Analysis was accomplished through GraphPad (Prism, 8.0, San Diego, USA) software.

Results and discussion

Experimental results

The results imply that the quadratic model was the best appropriate model for all responses (data not shown). According to the ANOVA outcomes, R^2 was 0/9147–0/9755, implying the high accuracy of the second-order polynomial model (Table 1). Furthermore, the lack of fit was not remarkable for all the models at a 5% significance level, which implies that the models were suitable forecasters of the dependent variables (data not shown). The predicted equations for each variable are depicted in Table 1.

Optimization of W/O/W double emulsions with vitamin D

Zeta potential

Zeta potential is a sensitive parameter that gives information about the electrical characteristics of the double-emulsion formulation. In general, the zeta potential is applied to show the strength of electrostatic interaction. The electrostatic

Table 1 Analysis of variances (ANOVA) of the models

Source	Mean	Std.Dev	CV	R^2	Adjusted R^2	Predicted Equation
SPAN index	2.70	0.0798	2.96	0.9755	0.9534	$y = 2.39 + 0.226x_1 + 0.127x_3 + 0.575x_1^2$
Zeta potential (mV)	-33.11	4.05	12.23	0.9147	0.8379	$y = -41.49 - 3.96x_1 - 3.98x_2 + 4.38x_3 + 13.02x_1^2$
Phase separation (%)	9.39	1.03	10.94	0.9581	0.9203	$y = 6.66 - 1.72x_1 - 1.17x_2 + 2.47x_3 - 0.8875x_1x_3 + 4.35x_1^2$
Interfacial tension (mN/m)	7.95	1.01	12.76	0.9161	0.8406	$y = 5.96 - 0.99x_1 - 0.87x_2 + 1.38x_3 + 3.64x_1^2$
Encapsulation Efficiency of vitamin D (%)	69.77	6.13	8.79	0.9450	0.8955	$y = 86.40 + 10.18x_1 + 1.56x_3 - 29.61x_1^2$

repulsion between droplets is enhanced with an increase in the absolute potential magnitude. So, it is an essential indicator of emulsion stability (Wang et al. 2022b).

The effects of zeta-potential on stability of emulsions is well explained by DLVO (Derjaguin–Landau–Verwey–Overbeek) theory, which affirmed that stability of colloidal systems related to the balance between the different forces acting on the interface. These are composed of electrostatic repulsive forces and van der Waals attractive forces owing to the surface charge. High zeta-potential, either positive or negative, is generally necessary to ensure stability. Generally, systems with zeta potential $> \pm 30$ mV are considered pharmaceutically stable (Sharma et al. 2014).

The measured zeta potential for various formulations of double emulsions was between -17.8 and -46.6 mV. The highest zeta potential (-17.8mV) belonged to the sample containing no flaxseed mucilage, 0.1% tween 80 and 20 ml external water phase content. According to the results, flaxseed mucilage content, Tween 80 content and the amount of external water phase have a remarkable impact on the zeta potential of double emulsion ($p < 0.05$). Flaxseed mucilage levels increasing (up to 0.75%) resulted in a decrease in the zeta potential, and a further increase in flaxseed mucilage content causes increased zeta potential (Fig. 1A). A slight decreasing trend in zeta potential was recorded as the content of tween 80 increased in the double emulsion formulation. In the case of external water phase content, observation showed an increase in the level of zeta potential as the amount of external water phase increased. Resistance behavior according to the zeta potential is described as follows: zeta potential = 0 to ± 5, Flocculation or coagulation happened, ± 10 to ± 30, Primitive instability. ± 30 to ± 40, medium stability. ± 40 to ± 60, fine resistance and $> \pm 60$, well resistance (Panigrahi et al. 2021).

The dispersion index (SPAN index)

The response surfaces in Fig. 1 (B) demonstrate the profile of the response (SPAN index) against the independent variables. As seen in Fig. 1 (B), an augment in flaxseed mucilage up to about 0.75% leads to a decrease in the SPAN index. Increasing the flaxseed mucilage content by more than 0.75% resulted in increasing the SPAN index of the double emulsion. Our results also matched the findings of Traynor et al. (2013), who reported that specific amounts of xanthan gum with sunflower oil in emulsions had a stabilizing effect, but higher concentrations resulted in a destabilizing impact and instability since of an accelerated creaming process owing to the promotion of droplet flocculation (Traynor et al. 2013).

The Tween 80% in double emulsion had no significant impact on the SPAN index of prepared double-emulsion, but the volume of the external phase had a remarkable impact on the SPAN index and accordingly, enhancing the volume of the external water phase caused a higher SPAN index in the double emulsion (Fig. 1B). The measured values of the SPAN index for various formulations were 2.25–3.42, which is in accordance with Keršienė et al. (2020), who reported the magnitude of SPAN index for empty double emulsion and loaded double emulsion (with various vitamins and black chokeberry pomace extract) were 2.588 and 4.650, respectively (Keršiene et al. 2020).

Interfacial tension

Analysis showed that all studied parameters (flaxseed mucilage content, Tween 80 content, and the amount of external water part) affect the interfacial tension of double emulsions. According to the results, the flaxseed mucilage content up to 0.75% caused diminish in interfacial tension, and the amount of flaxseed mucilage resulted in increasing this parameter. The relationship between the interfacial tension, and the amount of external water phase content is straight. As the amount of water increases, the level of interfacial tension also increases (Fig. 1C). Inversely, as the surfactant content increases, the interfacial tension decreases slightly. Leister et al. (2022) affirmed that the interfacial tension diminished with rising Tween 40 concentrations (Leister et al. 2022).

Phase separation (%)

Results showed that increasing the amount of flaxseed mucilage up to 0.75% causes a decrease in the percentage

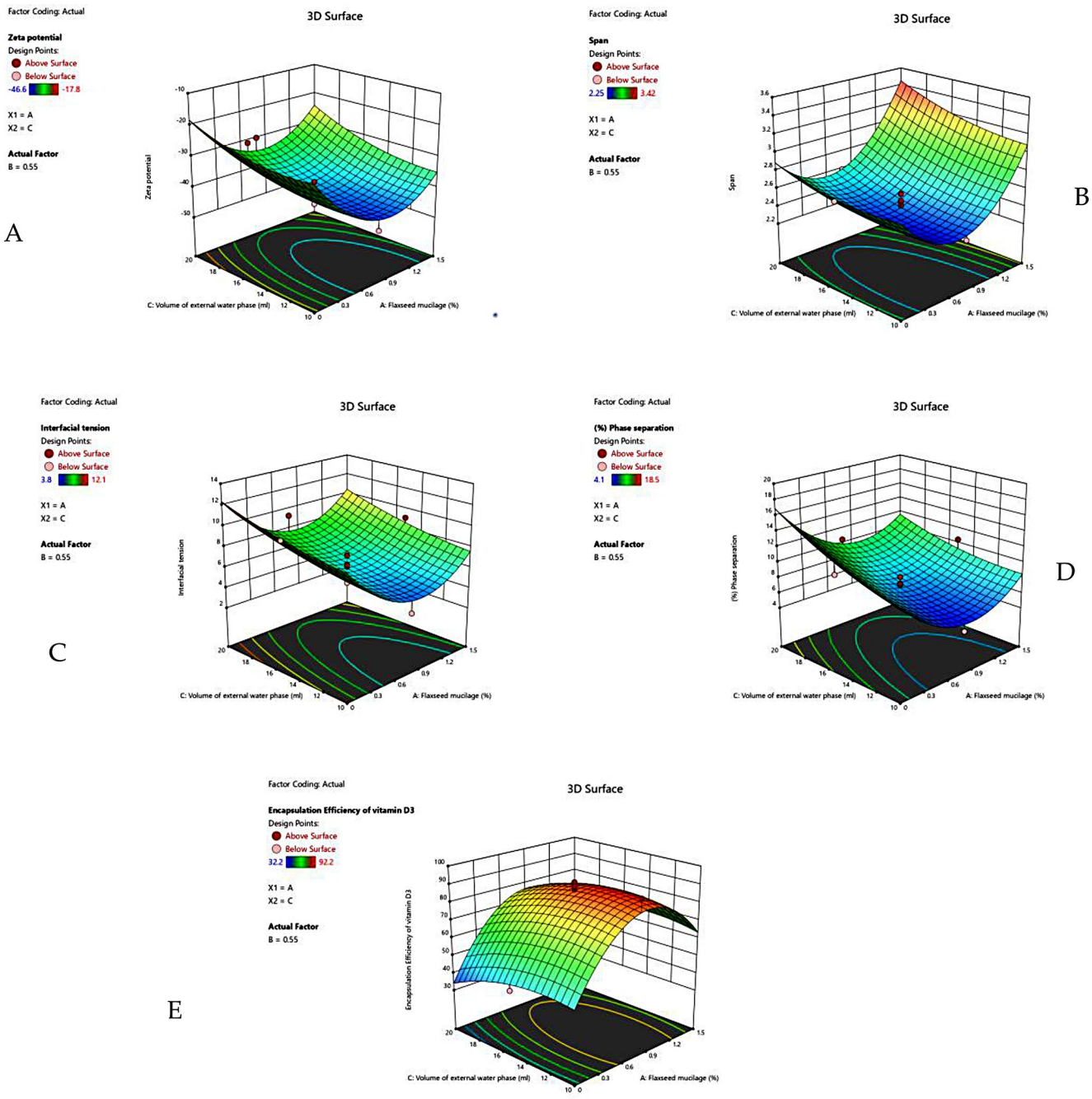

Fig. 1 The effect of flaxseed mucilage percentage and volume of external water phase on Zeta potential (**A**), SPAN index (**B**), Interfacial tension (**C**), phase separation (**D**) and Encapsulation efficiency of vitamin D (**E**)

of phase separation but the addition of more than 0.75% of flaxseed mucilage resulted in the enchantment of phase separation (Fig. 1D). Sapei et al. (2022) approved that the resistance of W/O/W emulsion is enhanced with the rising amount of soluble chitosan on the external aqueous part, and ascending this observation to the remarkably enhanced viscosity of the external aqueous part (Sapei et al. 2022). A similar explanation could cause the remarkable resistance of double emulsions containing flaxseed mucilage. Increasing the viscosity of the outer aqueous part through the inclusion of flaxseed mucilage resulted in retarding the speed of flocculation, or coalescence, of oil globules.

Observations revealed that the concentration of surfactant (Tween80) also had a significant effect on phase separation ($p < 0.05$), and an inverse relation was recorded between the content of the emulsifier and the percentage of phase separation ($p < 0.05$). Other research also confirmed the effect of the emulsifier amount on the resistance of double

emulsions. The external water phase content also significantly influenced the phase separation and resistance of double emulsions in the present study. There was a straight dependence between the external water phase amount and the percentage of phase separation (Fig. 1D). This implies that the resistance of double emulsion is reduced as the content of water in the outer phase increased. Yalçinöz & Erçelebi (2020) reported that addition of various hydrocolloids in (O/W) nano-emulsions enhances the stability of them and ascending this phenomenon to continuous phase viscosity improvement and/or delay of dispersed solid particle precipitation and oil droplets coalescence. Furthermore, the addition of sufficient magnitude of hydrocolloid to the aqueous phase of an emulsion prevents gravitational separation (creaming, sedimentation) by thickening the aqueous phase (Yalçinöz and Erçelebi 2022. Another reason for the increase in the stability of nanoemulsion via addition of flaxseed mucilage is related to the anionic nature of this hydrocolloid. Flaxseed mucilage presents anionic polysaccharides due to the presence of D-galacturonic acid which acts as hydrocolloid (de Filho et al. 2021). Xu et al. (2018) examined the effect of anionic diutan microbial polysaccharide on stability and rheology of O/W nano-emulsions and, reported increasing viscosity and better physical stability in the electrostatic repulsion and presence of non-absorbed polysaccharide molecules (Xu et al. 2018).

Encapsulation efficiency of vitamin D

The encapsulation efficiency of vitamin D was analyzed, and the results show flaxseed mucilage, and the amount of external water phase have a significant effect on encapsulation efficiency ($p < 0.05$). No significant effect of emulsifier content on encapsulation efficiency was observed ($p < 0.05$). The encapsulation efficiency of vitamin D was 92.2% (for a sample composed of 0.75 g flaxseed mucilage, 0.55% Tween 80, and 10 ml external water phase) ((Fig. 1E). Keršienė et al. (2020) approved the magnitude of encapsulation efficiency in double emulsion for vitamin D equal to 98.52 ± 10.43 (Keršiene et al. 2020).

The collected data analysis revealed that the amount of external water phase has a remarkable effect on the encapsulation efficiency of vitamin D and a reverse relationship between the two parameters was apparent (Fig. 1E). As the amount of external water phase increased, a reduction trend occured for vitamin D encapsulation efficiency.

Optimum circumstances of the double emulsion fabrication with vitamin D

The optimum circumstances based on the lowest SPAN index, zeta potential, phase separation and interfacial tension, and highest encapsulation efficiency were found to be equal to 0.75% flaxseed mucilage content, 0.55% Tween 80, and 15 mL external water phase volume. The predicted values were also confirmed by experiments, and there were no remarkable variations ($p < 0.05$). The desirability was equal to 0.966.

Fourier Transform Infrared Spectrometer (FTIR)

To investigate the chemical compatibility of optimized double emulsion, FTIR was applied to characterize the molecular behavior of flaxseed mucilage and double emulsion at optimal conditions. As shown in Fig. 2, flaxseed mucilage demonstrated a band at 3416.66 cm^{-1} ascribed to O-H stretching. It is demonstrated that a band at 1638.65 and a band at 1415.03 cm^{-1} due to the O-H bending were observed. The appearance of O-H in the FTIR of flaxseed mucilage might be attributed to the presence of α-L arabinose, and/or β-D-galactose in the structure of Rhamnogalacturonan I (Ochoa-Villarreal et al., 2014) which include about ~ 25% of flaxseed polysaccharides (Dzuvor et al. 2018). The appearance of O-H also could be attributed to the arabinose, galactose and xylose that are included in Arabinoxylan, and mak up 75% of flaxseed polysaccharides (Dzuvor et al. 2018). Another band was obvious at 1053.23 cm^{-1} attributed to the C-O stretching that could be ascending to the disaccharide repeating unit of α-(1,2)-D-Galacturonic Acid-α-(1,4)-L-Rhamnose (Dzuvor et al. 2018). In the case of double emulsion, O-H stretching band at 3404.10 cm^{-1}, and 3008.02 and 2953.50 cm^{-1} indicate C-H stretching. 1098.32 via the C-O stretching that might be ascending the structure of flaxseed mucilage and presence of Rhamnogalacturonan I and Rhamnogalacturonan I in its chemical structure (Dzuvor et al. 2018). The peak is prominent at 839.00 cm^{-1}, indicating C = C bending (IR Spectrum Table & Chart) (IR Spectrum Table & Chart, n.d.) that could be attributed to the chemical structure of vitamin D and the presence of the C = C bonds in its structure (Borel et al., 2014).

In vitro release of a double emulsion of vitamin D

In vitro release of a double emulsion of vitamin D eas measured and results showed in Fig. 3. Observations showed the percentage of the release of vitamin D in gastric conditions was significantly lower than the intestinal stage ($p < 0.05$). According to the results, the release of vitamin D in SIF condition has increasingly trended up to 105 min. A plateau was reached after the 100 min in the intestinal stage, with 96–98% of vitamin D released. The percentage of the release of vitamin D at the finished stage of the simulated intestinal phase (360 min) was 98%. Keršienė et al. (2020) pointed out that the magnitude of vitamin release under

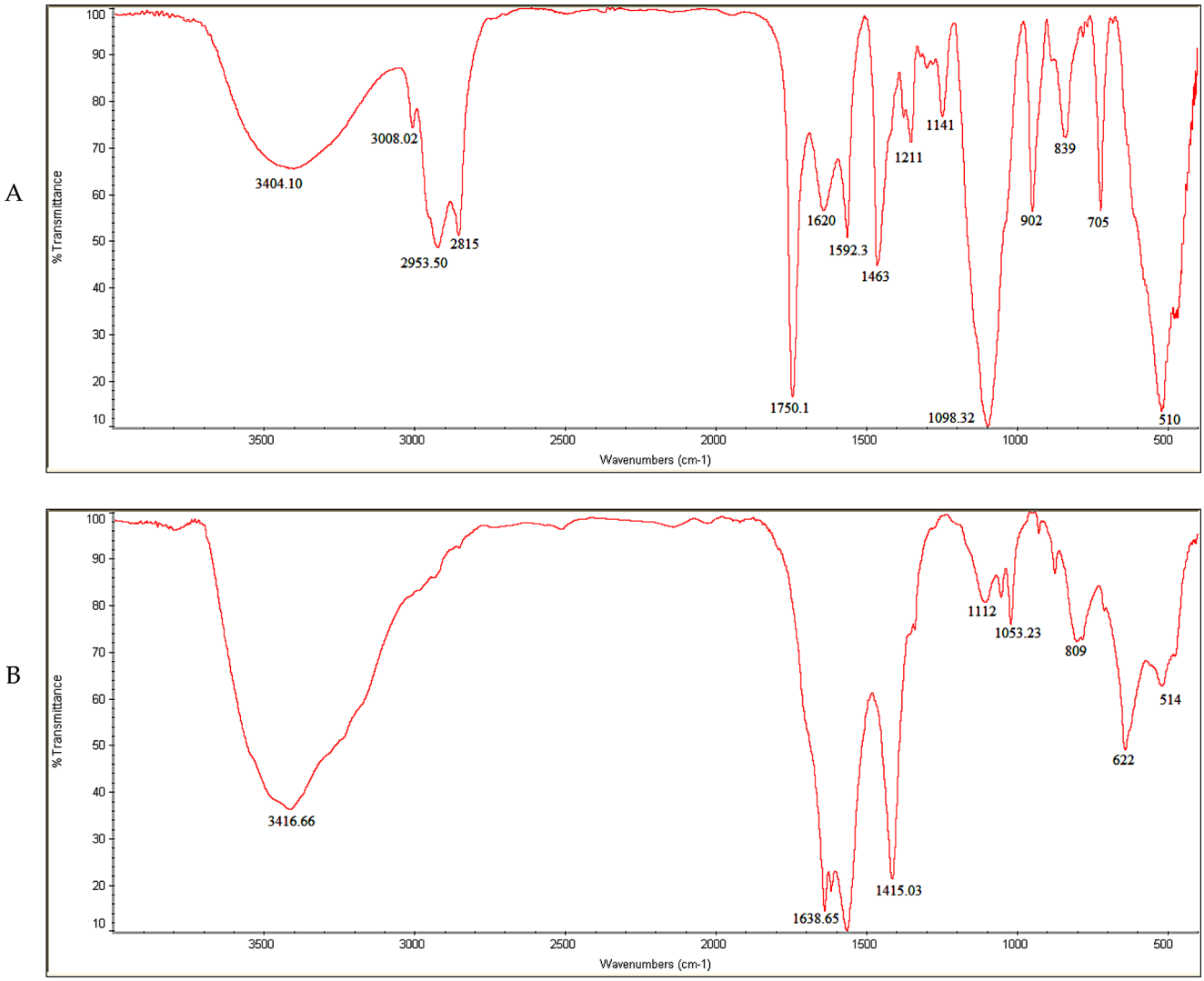

Fig. 2 Fourier Transform Infrared Spectrometer (FTIR) of double emulsion (**A**) Flaxseed mucilage (**B**)

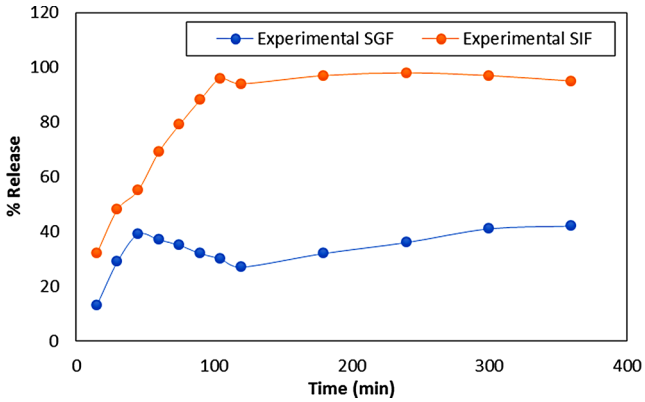

Fig. 3 Release of vitamin D from double emulsion as function of time during gastric (SGF) and intestinal (SIF) in vitro digestion

gastric circumstances was different, and for vitamin D was 70% (Keršiene et al. 2020). Comparing the R^2 value of various models for vitamin D release in gastric and intestinal simulated conditions showed that the R^2 value of the kopcha model was higher in both cases than other models, approving that the release followed the kopcha model kinetics in both gastric and intestinal simulated conditions (Table 2).

Vitamin D release is also not fitted by the zero-order model ($R^2 = 20.43$ for SGF and 21.29 for SIF) (Table 2). As depicted in Table 2, the Korsmeyer–Peppas release exponent (n) equals 0.2152 and 0.4092 in SGF and SIF circumstances, which approve that fickian diffusional release is the major mechanism. n is the diffusional exponent or drug release exponent (Osanlou et al. 2022). So, n magnitude is utilized to explain different release mechanisms; $n \leq 0.45$: a classical Fickian diffusion controlled. $n = 0.89$: non-Fickian, zero-order release. $n > 0.89$: super case II (increases

Table 2 Kinetic parameters of vitamin D release in SGF and SIF

	Vitamin D in SGF		Vitamin D in SIF	
Zero order model	K_0	0.289	K_0	0.1569
	R^2	20.43	R^2	21.29
First order model	K_1	0.1725	K_1	0.5596
	R^2	77.24	R^2	83.09
Kopcha model	A	0.073	A	0.5017
	B	-0.0019	B	-0.0052
	R^2	91.18	R^2	98.44
Korsmeyer-peppas model	k_{kp}	0.4211	k_{kp}	0.3959
	n	0.2152	n	0.4092
	R^2	88.65	R^2	80.91

Table 3 The magnitude of vitamin D (5 µg / 100 g) during storage at – 22 ºC

Storage days	Vitamin D content (5 µg / 100 g)
0	5 ± 0.01^a
7	4.9 ± 0.1^a
14	4.9 ± 0.1^a
28	4.9 ± 0.1^a

Table 4 In vitro release of vitamin D in simulated gastrointestinal condition

Simulated gastrointestinal condition	Released of vitamin D after digestion (µg / 100 g)
Oral	0.05 ± 0.01 µg/ 100 g
Stomach	0.19 ± 0.01 µg/100 g
Intestinal	4.74 ± 0.01 µg/100 g

plasticization at the relaxing boundary) release. $0.45 < n < 0.89$: both phenomena (diffusion and polymer relaxation) are included (anomalous transport).

Storage stability of vitamin D in ice cream samples

The percentage of vitamin D retention in hazelnut milk ice cream, including emulsified vitamin D was assessed. Accordingly, there was no remarkable variation in the magnitude of vitamin D retention during 28 days of storage ($p>0.05$) (Table 3). These outcomes are consistent with the report of Kazmi et al. (2007) that affirmed no degradation in vitamin D in ice cream samples on 0–4 weeks of storage at -25 °C and attributed this phenomenon to the low storage temperature, which likely played a remarkable function in the resistance of vitamin D in ice cream as any reaction corresponds to its degradation would be remarkably slowed down (Kazmi et al. 2007).

In vitro gastrointestinal stability of vitamin D in ice cream samples

The content of vitamin D in enriched hazelnut ice cream samples before and within simulated in vitro digestion was measured and, the results are shown in the Table 4. Accordingly, the initial magnitude of vitamin D (before in vitro simulated digestion was 4.9 ± 0.1 µg/ 100 g). In the present study, emulsified vitamin D was added before pasteurization, and there was no loss in vitamin D\ content after pasteurization. There were no remarkable variations between the added vitamin D before and after pasteurization ($p>0.05$). Esmaeili et al. (2022) reported no significant loss in vitamin D after milk pasteurization (Esmaeili et al. 2022).

At various stages of simulated gastrointestinal digestion, the magnitude of vitamin D was changed, whereas in oral in vitro circumstances, there was no significant change in vitamin D in the samples ($p>0.05$). The measured released content of vitamin D after the simulated oral condition was equal to 0.05 ± 0.01 µg/ 100 g, and there was no remarkable release of vitamin D occurred in this stage. These results could be ascending to the short exposure time and the neutral pH (equal to 7.0) in simulated oral circumstances (Wong et al. 2021).

Observations showed that in an in vitro simulated stomach condition, the measured content of released vitamin D was 0.19 ± 0.01 µg/100 g (about 4% of the initial vitamin D before digestion), which implies good protection of double emulsion structure in these circumstances. In an in vitro small intestinal condition, the measured vitamin D was 4.74 ± 0.01 µg/100 g, which implies about 94.8% of vitamin D was released in this condition. Obtained results imply that the controlled release of emulsified vitamin D occurred in vitro circumstances. This result is inconsistent with Park et al. (2017), who reported that the percentage of the release of vitamin D in simulated stomach conditions was 4%. In the simulated intestinal fluid, it was 90% (Park et al. 2017).

Sensory evaluation

A group (10 panelists) included regular consumers of hazelnut and dairy products, and assessed the various organoleptic properties (taste, preference, appearance and consistency) of samples by hedonic approach with nine points. Figure 4; Table 5 show the acceptance outcomes obtained for the control and fortified samples, and the acceptance of fortified samples after reading the text (after performing the Text Highlighting). According to the results, there were no remarkable variations between the control as well as the fortified hazelnut ice cream sample in various properties (taste, preference, appearance and consistency) ($p>0.05$), but the purchase intention of control and enriched samples showed

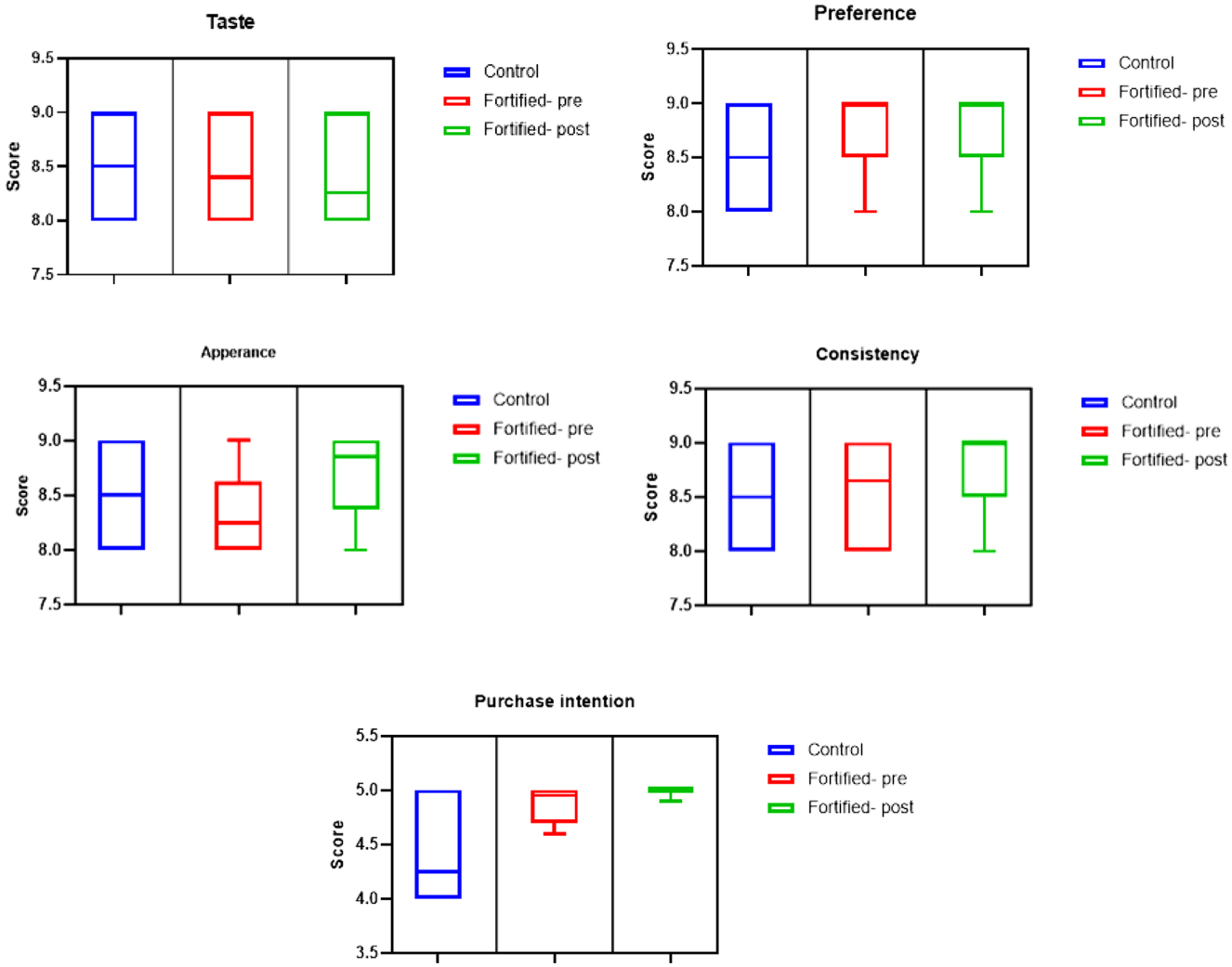

Fig. 4 Sensory acceptance of control (C) and Fortified (F) samples without information (Fortified- pre); and after read the text (Fortified-post)

Table 5 Comparison of the acceptance results obtained for control (C) and fortified (F) samples pre and post reading the text

Sensory attribute	Comparison	*p* value
Taste	C× F	0.652^{ns}
preference	C× F	0.168^{ns}
appearance	C× F	0.168^{ns}
consistency	C× F	0.343^{ns}
Purchase intention	C× F	0.006^{a}
Taste	F-pre × F-post	1.0^{ns}
preference	F-pre × F-post	0.758^{ns}
appearance	F-pre × F-post	0.161^{ns}
consistency	F-pre × F-post	0.129^{ns}
Purchase intention	F-pre × F-post	0.03^{a}

^{a} Significative difference and ^{ns} No significant difference at 5% of probability by Wilcoxon test; C: Fortified (F) samples without information (F- pre); and after read the text (F-post)

a remarkable variation ($p = 0.006$) (with mean equal to 4.41 and 4.86, respectively).

After reading the text highlighting, there was no significant difference in all attributes of fortified hazelnut ice cream samples (p >0.05), but a significant difference was observed between purchase intention before and after reading the text highlighting ($p = 0.03$) (with a mean equal to 4.87 and 4.99, respectively) (Table 5).

Conclusion

The focus of this research was to produce a water-oil-water double emulsion stabilized by flaxseed mucilage to protect vitamin D. Investigation showed that the flaxseed mucilage content and the volume of the external water phase have a remarkable impact on the SPAN index. Measurements illustrated that the flaxseed mucilage and Tween 80% and the volume of water in the external phase had a significant

effect on the zeta potential, interfacial tension, and phase separation (%)($P < 0.05$). The encapsulation efficiency of vitamin D was 92.2% (for a sample composed of 0.75 g flaxseed mucilage, 0.55% Tween80, and 15 ml external water phases). Observations showed the highest vitamin release rate occurred at simulated intestinal conditions for emulsified vitamin D. Observations affirmed that there were no remarkable variations in the magnitude of vitamin D retention during 28 days of storage at -22 °C ($p>0.05$). The content of vitamin D in fortified hazelnut ice cream samples showed that the amount of released vitamin D during in vitro simulated stomach and simulated small intestinal conditions was about 4% and 94.8% of the initial content of vitamin D, respectively. Sensory evaluation by an approach using text highlighting technique implies that there were no remarkable variations between control and fortified hazelnut ice cream samples in various properties but the purchase intention of control and fortified samples depicted remarkable variations ($p < 0.05$). After reading the text highlighting, there were no remarkable variations in all attributes of fortified hazelnut ice cream samples ($p > 0.05$) but a remarkable variation was observed between purchase intention before and after reading the text highlighting ($p = 0.03$).

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Author contributions Supervision: ZD, funding acquisition: ZD, conceptualization: ZD, software: ZD, M.H.H.KH. methodology: ZD, Z.D, data curation: Z.D, M.H.H.KH. validation: ZD, formal analysis: ZD, M.H.H.KH. writing—original draft: ZD,;writing—review and editing, ZD, M.H.H.KH, B.G.

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Data availability Data available on request from the authors.

Code Availability It is not applicable.

Declarations

Ethics approval 'Not applicable'.

Consent to participate Consent to participate is not applicable.

Consent for publication It is not applicable.

Conflict of interest There is no conflict of interest to declare.

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