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Effect of Fortification Dairy Products with Wheat Germ Oil for Extending Shelf Life

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ABSTRACT

The core portion of the wheat grain that results from the milling process is called the germ. Therefore, the study's goal was to create alcoholic, water-based extracts and oil of wheat germ and to produce cream using a major proportion of the extracts and oil. The oil was extracted by supercritical fluid extraction (SFE) using CO_2 a solvent. Our experiment used a GC-MS, or gas chromatography-mass spectrometry, to examine and determine the WGO components. Phytochemical screening detected glycosides, alkaloids, phenols, coumarin, saponin, steroids, terpenoids, flavonoids in the wheat germ extracts and oil. Additionally, the study examined the effects of various extracts on the antioxidants, free fatty acids, and organoleptic characteristics of freshly made and stored cream samples for ten days at 8°C ± 1. It was found that WGO extract exerted stronger antioxidant activity than ethanol and water extracts. This research showed that the incorporation of oil from wheat germ at a concentration of 0.4% could extend samples of cream that can be stored for up to 10 days at 8 ± 1°C. This extension in shelf life was achieved without the need for any chemical preservatives, while still maintaining acceptable taste, flavor, and texture. This study found that WGO and extracts offer a substitute for artificial preservatives in the food industry. It will also assist researchers in identifying important aspects of functional dairy products that many others **could not** investigate.

تأثير تدعيم منتجات الالبان بزيت جنين القمح في اطالة العمر الخزني

نهلة طارق خالد، شذى جميل الخالدي، شيماء رفعت خيري، شيماء سعدي لفتة
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الخلاصة

الجزء الأساسي من حبة القمح الناتج عن عملية الطحن يسمى جنين القمح . كان هدف الدراسة هو تحضير مستخلصات الكحولي والمائي والزيت من جنين القمح، وإنتاج الكريمة (القشدة) وتدعيمها بنسبة من المستخلصات والزيت. استخلص الزيت بطريقة السوائل الحرجة (SFE) باستخدام CO_2 كمذيب. استخدمت تجربتنا كروماتوغرافيا الغاز-مطياف الكتلةGC-MS لفحص وتحديد مكونات زيت جنين القمح. كشف الفحص الكيميائي النباتي عن الكلايكوسيدات والقلويدات والفينولات والكومارين والسابونين والستيرويدات والتربينويدات والفلافونويدات في مستخلصات جنين القمح والزيت. وعلاوة على ذلك، فحصت الدراسة تأثيرات المستخلصات المختلفة على مضادات الأكسدة والأحماض الدهنية الحرة والخصائص الحسية لعينات الكريمة الطازجة والمُخزنة لمدة عشرة أيام عند درجة حرارة 8 درجة مئوية ± 1. وقد وجد أن مستخلص زيت جنين القمح أظهر نشاطًا مضادًا للأكسدة أقوى من المستخلصات الإيثانولي والمائية ،وأظهرت الدراسة أن تدعيم الكريم بنسبة 0,4٪ زيت جنين القمح يمكن أن يطيل مدة صلاحية عينات الكريمة بحيث يمكن تخزينها لمدة تصل إلى 10 أيام عند 8 ± 1 درجة مئوية دون الحاجة إلى أي مواد حافظة كيميائية، مع الحفاظ على المذاق والنكهة والملمس المقبولين. ووجدت هذه الدراسة أن زيت جنين القمح ومستخلصاته يقدمان بديلاً للمواد الحافظة الاصطناعية في صناعة الأغذية. كما يساعد الباحثين في تحديد الجوانب المهمة لمنتجات الألبان الوظيفية التي لم تدرس لحد الان

الكلمات المفتاحية: الاستقرار التأكسدي؛ الفحص الكيميائي النباتي؛ تطوير المنتج؛ استخلاص السوائل فوق الحرجة؛ زيت جنين القمح

INTRODUCTION

The cream is one of the most important dairy products. The fat content could be considered as the main compound of the cream around 20.0 % to 85.0 %. The cream is classified into three types depending on its fat content: light cream, medium cream and heavy cream and Plastic cream. The light cream has a low-fat content of 20-25% milk fat and is also called market cream, including table, whereas the heavy cream has approximately 30-40% milk fat, sterilized, and coffee cream and Plastic cream 65-85% milk fat (Atanu, 2018).

A complex mechanism that combines a series of enzymatic or non-enzymatic reactions is called lipid oxidation. Free radical formation starts the process, which is followed by an oxygen attack and a shift in the location of the double bond in the lipid structure. It breaks down the lipids in the membrane and produces a variety of oxidation products, including ethers, alcohols, ketones, alkanes, and aldehydes (Repetto *et al.* ., 2012; Abdulameer & Hussein, 2023; AL-KHALIFA *et al.* .,2023).

Today, the dairy industry has advanced in many countries by adding non-structural ingredients such as fresh fruits, vegetables, and plant proteins. Numerous plants and herbs, including Myrtus, beetroot, cardamom, pomegranate, and thyme (Thymus vulgaris), have demonstrated possible health advantages, including antioxidant qualities (Nahla *et al.* , 2018; Wisam *et al.* ., 2018; Nurcholis *et al.* ., 2021; Lazeeza, 2021; Maha *et al.* ., 2024; Ali *et al.* ., 2024). To increase total phenolic antibacterial and antioxidant properties of dairy products, herbal extracts have been employed as fortifying agents (Granato *et al.* ., 2018; Awda *et al.* ., 2019; Khudair *et al.* ., 2024). The oxidation mechanism is inhibited by antioxidants.

Antioxidants establish a defense system that removes oxidative stressors and stops cell death. They are separated into primary and secondary antioxidants based on how they function in the mechanism. Primary antioxidants give lipid radicals hydrogen, which breaks the chain of free radicals (Wsowicz *et al.* ., 2004; Nike *et al.* ., 2005).

Wsowicz *et al.* . (2004) state that $ROO\bullet + AH \rightarrow ROOH + A\bullet$

Chain breaking does not directly involve secondary antioxidants. Instead, by scavenging metals and oxygen, reducing metals, holding singlet oxygen, and recovering the main antioxidants, it stops oxidation (Wsowicz *et al.* ., 2004). Because of its high nutritional and health value, wheat germ—which makes up 2.5–3.5% of the wheat grain's weight—has great significance as a functional food. Because of its composition of fats, carbohydrates (especially sucrose), fiber, mineral salts (like potassium, magnesium, zinc, and phosphorus), and essential vitamins from the B group and vitamin E, it plays a crucial role in human nutrition.

Wheat germ oil (WGO), a by-product of grain milling can be extracted using a variety of methods. WGO can be processed more effectively with the use of contemporary techniques like supercritical fluid fractionation, molecular distillation, and other recently developed techniques. The content of WGO consists of different sectors and they are long-chain fatty acids (LCFAs), Hydrocarbons, pigments, sterols, 4-methyl sterols, triterpenols, alcohols, esters, alkenes, aldehydes, tocopherols, n-alkanols, glycolipids, phospholipids, and volatile substances. The main fatty acid in WGO is linoleic acid, which makes up 42–59% of the total triglyceride content. Oleic and palmitic acids (16:0 and 18:1, respectively) are the subsequent major types of fatty acids in this case. WGO usually has less than 2% saturated fatty acid found in fat stearic acid. It is in the vitamin E family, and it is rich in the tocopherols, both alpha-tocopherol and beta-tocopherol, which have identified share of health benefits. It is being used in medicine, cosmetics, agricultural and food industry (Ketan *et al.* ., 2020). Because of its many beneficial qualities, WGO is widely used in the medicine, food, pharmaceutical, cosmetic, and agricultural industries. The WGO is going to be used for various market segments, the product includes vitamins and dietetics, and the animal feed uses WGO with biological insect control methods as well as weak cardiac/circulatory issues treatment (Özcan *et al.* ., 2013, Al-Abody & Al-Hamied, 2022).The study conducted by Jabar and Al-Mosawi (2018) showed that the wheat germ has the property of biostimulant in preparation of therapeutic lactoferments foods due to its action in increasing the numbers of probiotic bacteria, and it’s as well a positive influence on improving sensory and nutritional characteristics.

The impact of wheat germ extract on probiotic-derived exogenous polysaccharide synthesis and prepared several therapeutic fermented milk products using single and mixed starters for both *Lactobacillus acidophilus* and *Lactobacillus plantarum* (Abd Al-Jabar & Al-Mosawi, 2018)

According to Khalid *et al.* . (2021), when wheat germ extracts were added to cheese after heat treatment, the cheese's solids content and hardness increased in comparison to the control treatment. The taste, flavor, and texture characteristics of the cheese product were also found to be acceptable after storage at 8^{o}C for 21 days.

Researchers Nahla & Makarim (2018) and Tariq *et al.* . (2023) discovered that adding wheat germ to cow's milk white cheese significantly altered its fatty acids and chemical composition. The findings indicated an improvement in cheese yield, a decrease in coagulation time, and an increase in fatty acids, ash, and protein levels.

This study aims to develop dairy production technology and explore the antioxidant and sensory attributes of fortified dairy products with wheat germ. Specifically, the focus was on cream fortified with wheat germ aqueous and alcoholic extracts, as well as oil which contain active compounds (antioxidants) and help prevent the oxidation of the cream fat and extend the shelf life of the product. It is known that the duration of using the cream does not exceed three days, so it was important to extend the storage period of the cream to ten days or more.

MATERIALS AND METHODS

Materials

The college dairy plant connected to the Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, provided the fresh cream (light cream) used in this investigation. The General Company for Cereal Manufacturing, Ministry of Trade, Baghdad, Iraq, provided the local wheat germ. An electric mill was used to grind the wheat germ to prepare the wheat germ for extraction.

Wheat germ extraction of oil

The extraction with supercritical CO_2: Approximately 100 grams of wheat germ samples were placed into an aluminum cylindrical sample holder with 23 mm diameter and 15 cm height for the extraction procedure. The extractor had a volume of 150 mL, and the separators had an estimated volume of 50 mL. More information regarding the apparatus can be found in the studies of Jozwiak *et al.* . (2013). The pressure for oil recovery in the experiments was achieved using a single separator operating at 250 bar, while a temperature of 40°C was selected as the norm for the supercritical fluid extraction (SFE) experiments (Piras *et al.* ., 2009).CO_2 flow rate used in the experiment averaged around 60 dm^3 h^{-1} pressure. After extraction, the obtained sample was subjected to analysis utilizing a Shimadzu QP2000A instrument from Kyoto, Japan, for Gas Chromatography-Mass Spectrometry (GC-MS). This analysis was conducted to determine the contents of the sample.

Oil extraction with hexane : The purpose of extracting wheat germ oil using hexane is to compare the extraction ratios (oil) between the solvent extraction method (hexane) and the CO_2 extraction method. Each stabilized wheat germ sample weighing 300 g was subjected to extraction using hexane (Merck, Darmstadt, Germany) in a ratio of 1:10 (mass to volume) employing the Soxhlet extraction technique for roughly twenty hours. The resulting extract was passed through a Büchner funnel equipped with number 1 filter paper from Maidstone, UK's Whatman International Ltd, to facilitate filtration. Under the same circumstances, the extraction procedure was carried out twice. A rotary evaporator (Laborota 4000; Heidolph Schwabach, Germany) was used to evaporate the solvent at 40 °C. After that, the resultant oil was put in a glass container and kept at 4 °C until it was time for additional examination.

2.3 Analysis using Gas Chromatography-Mass Spectrophotometry (GC-MS) : GC-MS analysis was used to determine the active compounds in the wheat germ oil CO_2 extract. An Agilent Technologies 7820A GC-MS Gas Chromatograph with an autosampler and a 5977MSD Mass Selective Detector were used for the analysis. The chromatographic column was an Agilent HP 5 column (30 m length × 250 µm inner diameter × 0.25 µm film thickness), with high-purity helium (He) was used as the carrier gas in. The injector temperature was 250 ◦C. The source temperature of MS was set at 250 ˚C and the Quad temperature was set at 150 ˚.The heating program was set at an initial temperature of 250 ˚C (held for 1 min), then increased to 300 ˚C at a rate of 10 ◦C/min held for 1 min. The high temperature was maintained for 230 min until the analysis was completed. The scan range was set at m/z 25-1000 mass ranges at 70 eV electron energy, with a solvent delay of 3 min.

A 1 µL sample of WGO was injected directly into the GC-MS system via autosampler. To identify the eluting compounds, a mass spectral library was employed, which entailed contrasting the compounds' mass spectra with those that were kept in the database (Zargar *et al.* .,2023). The database contains an extensive collection of over 163,000 electron impact (EI) mass spectra for reference (Jing, *et al.* ., 2022)**.**

Preparation of extracts

Alcoholic extract

To make crude extracts from wheat germ, 95% ethanol was adopted as a solvent. In a conical flask, 50 grams of 500 mL of ethanol and wheat germ were combined and then refrigerated for a full day. A magnetic stirrer was used to agitate the mixture at 6000 cycles per minute

and filtered afterwards. The final result of the extraction was collected and stored in bottles sealed tight and kept in a refrigerator until it was prepared for analysis, at 4°C (Abaza, *et al.* ., 2011).

Aqueous extract

In a conical flask, 10g of wheat germ (it was a primary kind of enzyme activating in the process) distilled water, and 100 mL of a magnetic stirrer were used to mix the mixture. To heat it to 100°C for one hour stirring. During the subsequent steps, the solution was heated up and boiled to eliminate the dust, soil and dirt and the centrifuge, which is known for its 6000 RPM velocity, was used to clean with a high rate of production. Consequently, the mission is that you re-take the nutrient solution from the plant, put it in the refrigerator, and then the solution will be administered the next day (Khalid *et al.* ., 2021).

Qualitative phytochemical analysis

The standard protocols were employed to conduct qualitative analysis of phytochemicals in different wheat germ extracts using three distinct solvents: oil, alcoholic, and aqueous.

Test for glycosides

Benedict's reagent was made by dissolving 100 grams of sodium carbonate monohydrate and 137 grams of sodium citrate in 800 milliliters of distilled water. After filtering, the solution was added to the filtrate. Cupric sulphate solution (17.3) gm in (100) milliliters of distilled water; Use distilled water to bring the volume up to 1000 milliliters. This detector produces a red precipitate at the bottom, indicating the presence of glycosidic compounds. When 5 ml of Benedict's reagent is mixed with 1 ml of aqueous plant extract, the presence of glycosides is confirmed by the formation of a red precipitate (Noman & Suliman 2023).

Test for alkaloids

Mayer's reagent is prepared by dissolving potassium iodide (5.00 g) and mercuric chloride (1.36 g) in 100.0 mL of water to create a new solution. When a neutral or slightly acidic solution containing alkaloids is treated with Mayer's reagent, a cream-colored precipitate is formed. 1.36 g of mercuric chloride and 5 g of potassium iodide are dissolved in 60 mL of distilled water and 20 mL of distilled water, respectively, to prepare Mayer's reagent. After mixing the two solutions, distilled water is added to bring the volume down to 100 mL.

0.5 grams of the extract were dissolved in 10 milliliters of a diluted hydrochloric acid solution to check for the presence of alkaloids (0.1 N) and subsequently filtered. The resulting filtrate was utilized for the alkaloid presence test.

The obtained filtrates were subjected to treatment with Mayer's reagent. The emergence of a yellow cream-coloured precipitate shows the formation of alkaloids, reports Swarup and Rajasekharan (2023).

Test for phenols

A 1% ferric chloride reagent was used conforming to the suggested method by Jagaba and Companions (2018). This solution was prepared by weighing (1) gm of ferric chloride placing it in a graduated cylinder, and finally completing the volume up to 100 mL. Phenols were detected with this colour solution of bluish-green.

Test for coumarin

The coumarin molecule in the alcoholic extract of the plant was traced by preparing 5 mL of such a test tube. At the top of the test tube was a filter paper that was immersed in a dilute sodium hydroxide solution. The tube was heated for five minutes in a hot water bath. The filter paper was put under UV light with a transilluminator after that. The presence of coumarin has been demonstrated by the observation of yellow-green color on the filter paper as shown in the work done by Suriyakala *et al.* . (2022).

Test for Saponins

A small package of the drug (powdered residue), was immersed in 95% ethanol. Then, the filtration followed and in one test tube ten milliliters of distilled water were mixed with 2.5 milliliters of pure solution. The test tube was carefully wrapped and then shaken vigorously for

about 30 seconds. On the other way, the tube was kept horizontal for half an hour without any movement. Once saponin is added to the solution that was initially clear, the frothing foam formation in the test tube is proof of its presence. Shake the test tube vigorously to witness the foam's persistence for a long duration of time.

Test for terpenoid and steroid

One milliliter of the crude extract was mixed with two milliliters of chloroform. The mixture was then supplemented with 1 mL of anhydrous acetic acid and 1 mL of concentrated H_2SO_4. A positive terpenoid test result is indicated by the presence of brown, while the presence of steroids in the sample is indicated by the appearance of blue (Sofowora, 1993; Nuraskin *et al.* ., 2020).

Test for flavonoid

A 2 cm^3 portion of the extract was combined with distilled water in a 1:4 ratio for the ferric chloride test. The mixture was then mixed with a few drops of a 10% ferric chloride ($FeCl_3$) solution. When flavonoids are present, a green or blue solution will appear (Sofowora, 1993).

Analytical determinations of wheat germ oil

The AOCS official method Ca5a-40 (1997) was employed to determine the levels of Peroxide value (PV) and free fatty acids.

Milk separation into cream and skim milk

A pilot plant separator (Elecrem separator, model 1G, 6400 rpm, Bonanza Industries, Inc., Calgary, Alberta, Canada) was used to separate the raw milk, which came from the dairy plant of the College of Agricultural Engineering Sciences at the University of Baghdad.
This separation process resulted in the production of cream with a milk fat content ranging from 30% to 35%, as well as skim milk.

Creams that were obtained through the separation process containing 30 to 33% milk fat were adjusted at either 49°C or 55°C by incorporating the appropriate amount of skim milk that was collected during the separation process. Each cream underwent 30 minutes of vat pasteurization at 68.3°C. Following pasteurization, the creams were cooled down to a temperature of 13°C using an ice bath, as described in the research carried out by Scott *et al.* . (2003). The cooled creams were then divided into seven separate batches. The control cream treatment (A) was prepared without the addition of wheat germ extracts and oil. The remaining six treatments (B1, B2) were created by adding 0.2% and 0.4% oil to the cream by weight, 0.2% and 0.4 % aqueous extract (C1, C2), and 0.2% and 0.4 % ethanolic extract (D1, D2), respectively, and storage in the cooling at 8ºC for 0, 5, and 10 days.

Sensory Evaluation

The creams were subjected to an assessment of their sensory attributes within a time frame of ten days following storage at a refrigerated temperature of 4°C. The treated cream's sensory assessment was carried out by a panel consisting of experienced professionals (n=10) from The Department of Food Science at the University of Baghdad, individuals were chosen predicated upon their previous involvement in sensory assessments of dairy, using the In/Out

Method of Specification to determine if the creams met predetermined standards for quality. Before being presented to the panellists, the samples were randomly selected and identified using letter codes. Seven samples in all (20 mL each in 30-gram portion-size plastic cups) were provided to the panellists, one for each cream formulation. Throughout the sensory evaluation, the sample temperature was kept between 3 and 4°C. The evaluation of various properties was carried out based on the provided samples. These properties included package shape (rated on a scale of 1 to 5 points), public acceptance (rated on a scale of 1 to 20 points), taste and flavor (rated on a scale of 1 to 45 points), body and texture (rated on a scale of 1 to 20 points), and color (rated on a scale of 1 to 10 points) (Ziablitseva *et al.* ., 2022).

Analysis of Statistics

SAS software version 9.3 was used to analyze the data (SAS Institute, 2012**).** The study which was based on the CRD aimed at identifying the factors that influence the given parameters and 0.05 was accepted as the critical level to test the level of importance of the attained statistical.

RESULTS AND DISCUSSION

The WGO yields were raised by 2.06% and 13% (w/w) with the use of supercritical CO_2 (SC-CO_2) and the solvent technique, respectively. Moreover, the picture shows that over 80% of the oil was recovered from underground. CO_2-COP_2 yield turned out to be pretty close to the yield of solvent extraction which is impressive. A comparative study was undertaken with two objectives which mainly focused on assessing the superiority of the oils produced through organic solvent (hexane) extraction and supercritical carbon dioxide (SC-CO_2) extraction. The oil from the organic solvent showed signs of non-selectivity contributing to a product that consists of some unwanted compounds, which shows that the organic solvent is less selective compared to CO_2. Utilizing supercritical fluids (SFE) with liquid and gas-like characteristics at temperatures and pressures above the critical point, supercritical fluid extraction (SFE) is an eco-friendly extraction method. These solvents are gases at room temperature and are typically non-toxic, in contrast to the most widely used solvents for oil extraction, such as hexane, methanol, and chloroform (Picot-Allain *et al.* . 2021). The parameters used during extraction, such as pressure, extraction time, temperature, solvent flow rate, and the use of co-solvents, must be modified when planning and refining the scCO2 extraction process. Similarly, it is crucial to reduce the particle size of the material to be extracted because doing so increases the extraction yield (Ahangari *et al.* . 2021).

The carbon dioxide is compressed to the desired pressure, heated, and pumped around the extraction and separation vessels. Supercritical CO2 dissolves the oil in the extractor and carries it to the separation vessel where the oil is separated by changing CO2 pressure and temperature. The oil can be directly fractionated in several fractions as the separation can be done in several steps at different conditions (Grigaliūnaitė & Ruiz-Méndez, 2023). Hence, the use of supercritical CO_2 for extraction offers advantages not only in reducing the energy required for extracting crude oil but also in eliminating or streamlining processing steps involved in refining

the extracted oil. The top 22 compounds found by gas chromatography-mass spectrometry (GC-MS) analysis of WGO are listed in Table 1. Fig. 1 displays the total ion chromatogram obtained from the analysis. The amount of the corresponding compound in the sample is directly proportional to the area under each peak in the chromatogram.

Table 1: The main components of WGO along with their chemical structure were determined using gas chromatography-mass spectrometry analysis.

No.Pk.	RT (min)	Area peak (%)	Compound	Intensity
1	3.227 ±0.51^{c}	1.05 ±0.08^{c}	2,5-Dihydroxyheptane	14428 ±235.9^{c}
2	3.405 ±0.46^{c}	11.05 ±0.76^{c}	1-(3-ethyloxiranl)	73872 ±507.1$^{a\ c}$
3	3.513 ±0.49^{c}	22.46 ±1.48^{a}	2-Aminoethanol	21335 ±266.2^{c}
4	3.863 ±0.76^{c}	12.82 ±0.84^{b}	2,4,6,8-Tetramethyl-1-undecane	84065 ±761.6$^{b\ c}$
5	4.582 ±0.65^{c}	3.45 ±0.42^{c}	6-Nitrohexan-2-o1	22382 ±167.9^{c}
6	4.792 ±0.77^{c}	4.18 ±0.55^{c}	Lyxitol, 1-o-hexyl-	90257 ±745.7^{c}
7	5.237 ±0.63^{c}	4.30 ±0.42^{c}	3-Methylpenta-1,4-diene-3-o1	105894 ±893.2^{cd}
8	6.872 ±0.78^{c}	3.02 ±0.29^{c}	Anethole	22608 ±317.6^{c}
9	11.841 ±0.85^{bc}	1.32 ±0.14^{c}	Trans-13-Octadecenoic acid	129357 ±1045.8^{c}
10	11.860 ±0.81^{ac}	0.65 ±0.25^{c}	13-Docosenoic acid, methyl ester,(z)-	185501 ±667.3^{c}
11	11.924 ±0.85^{c}	0.20 ±0.06^{c}	13-Docosenoic acid, methyl ester,(z)-	185501 ^{c}±705.8^{ce}
12	12.108 ±0.91^{c}	2.12 ±0.17^{c}	Cis-9-Hexadecanoic acid	105678 ±642.9^{c}
13	12.185 ±0.87^{c}	0.33 ±0.08^{c}	Cis-9-Hexadecanoic acid	105678 ±439.6^{c}
14	12.884 ±0.83^{c}	10.50 ±0.74^{a}	Methyl 18-methylnonadecanoate	166215 ±1267.9^{c}
15	14.227 ±0.92^{c}	4.30 ±0.36^{c}	Erucic acid	175491 ±1773.3^{cf}
16	14.691 ±0.77^{c}	4.86 ±0.55^{c}	Oleic acid	129336 ±861.7^{c}
17	15.079 ±0.89^{c}	2.86 ±0.15^{c}	Cis-Vaccenic acid	129339 ±749.2^{c}
18	15.289 ±0.92^{c}	2.85 ±0.18^{c}	Oleic acid	129336 ±644.2^{c}
19	15.429 ±0.97^{c}	1.61 ±0.05^{c}	Cis-13-Octadecenoic acid	129347 ±750.8
20	15.6152 ±1.08^{c}	1.81 ±0.08^{c}	Cis-13-Octadecenoic acid	129347 ±563.8^{c}
21	15.843 ±0.83^{c}	0.24 ±0.06^{c}	Oleic acid	129335 ±806.3^{c}
22	16.587 ±1.07^{c}	4.04 ±0.27^{c}	Oleic acid	129335 ±771.0^{c}
L.S.D.	3.461 *	2.759 *	---	405.912 *
		* (P≤0.05).		

Each column contains values (mean ± SD, n = 2) that are significantly ($p < 0.05$) different and are superscripted with different lowercase letters.

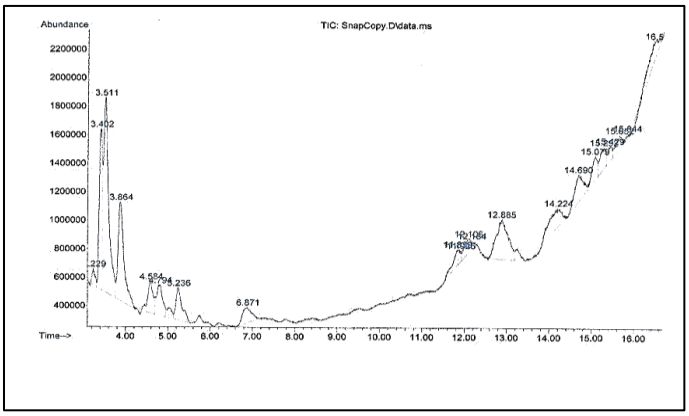

Figure 1. GC-MS Chromatogram of WGO

Table 1 demonstrates that extracts with higher content are linked to a greater presence of active ingredients. Specifically, Methyl 18-methylnonadecanoate (97%), Anethole (96%), Oleic acid (95%), Cis-Vaccenic acid (95%), Cis-13-Octadecenoic acid (95%), and Cis-9-Hexadecanoic acid (93%) were found to be the most abundant compounds, as indicated by their quality, retention duration, and peak regions in Fig. 1. Additionally, other fatty acids were also identified among the compounds present. According to Ramadan *et al.* ., (2024), among the compounds identified, oleic acid has antibacterial and antioxidant qualities.

Plants contain hexadecanoic acid, a fatty acid with a variety of biological functions, such as hypocholesterolemic and antioxidant effects (Siswadi and Saragih 2021). The stronger antioxidant activity of the WGO extracted by supercritical CO_2 may be explained by the higher levels of unsaturated fatty acids and total phenolic compounds in the oil. Unsaturated fatty acids, particularly oleic and erucic acids were abundant in WGO. These studies also reported similar findings (Piras *et al.* ., 2009; Özcan *et al.* ., 2013; Soliman, 2020).

The major active components were determined in the extracts and oils samples respectively and are shown in Table 2 below. The crude wheat germ extracts and oil samples were subjected to phytochemical screening and it was found that they contained several active elements like glycosides, alkaloids, phenols, coumarin, saponin, steroid, terpenoids, and flavonoids. Taking into account the interaction mechanism of these compounds, they revealed medical and physiological properties during their solvent extraction. Moreover, the water extract was shown to give a higher number of metabolites compared to the ethanolic and hexane extraction of wheat germ extracts. To View Table 2, one can observe that the solvent extract along with the oil of wheat germ contains Glycosides. These glycosides have been of particular interest because of their side effects like the cytokinin and cancer, talked about by Juszczak *et al.* . (2021). A study that How Hussein *et al.* . also (2018) conducted supports that the presence of glycosides as well as through primary biological analysis is in the spend of wheat residues. Although Table 2 shows the detected alkaloids in both solvent extracts and WGO, it doesn't state the levels of their concentration in comparison to each other. The alkaloids perform a couple of key tasks in plants. They protect the plants against attack from predators and alkaloids regulate

the growth of plants which has been highlighted by Heinrich *et al.* . (2021). As Heinrich and his team have suggested, alkaloids stand out through their features as anesthetics, cardioprotective, as well as for anti-inflammatory. In their work previously, Hussein *et al.* ., (2018) indicated that alkaloids are also present in wheat germ extracts.

Table 2 indicates that the aqueous extract had the highest concentration of phenolic compounds, surpassing the levels in the ethanolic and hexane extracts. Zhang *et al.* . (2022) suggest that High phenol content is closely linked to antioxidant activity since phenolic compounds play a major role in plants' antioxidant qualities. Phenols have one or more hydroxyl groups in their aromatic ring structure, enabling them to chelate metal cations, donate hydrogen atoms or electrons, and scavenge free radicals, as described by Costa *et al.* . (2021).

Coumarin belongs to the benzopyrone chemical class and has a vanilla-like fragrance. Unique physical and chemical properties make coumarin a promising class of bioactive heterocyclic compounds that occur naturally. Compounds containing the coumarin structure have an extensive range of pharmacological, natural, and physiological activities, which makes them important for use in farming, medicine, and the food industry. It was found that fungal growth can be inhibited by substituents connected to the coumarin core (Lončar *et al.* ., 2023). Coumarin was identified in this study in the two wheat germ extracts and oil (Table 2).

The presence of persistent foam during testing suggests the presence of saponins in the sample. Both the wheat germ extracts and its oil contain saponins, as indicated in Table 2. Saponins have been utilized as dietary supplements and have shown expectorant and anti-inflammatory properties, as noted by Shamsu and Abubakar (2016).

Among the large variety of solvent extracts and oils, there appears to be the presence of steroids and terpenoids in different quantities (Table 2). According to the information in Table 2, the shape of flavones is visible in any extract of wheat bran. According to the research results of (Labga *et al.* ., 2021), wheat germ had the highest concentration of total flavonoids. Flavonoids are bioactive compounds from plants and they are important for human health through numerous favorable effects. Theirs can be found mainly in the flowers, roots, and extracts that are created through fermentation (Hussein *et al.* ., 2018; Al-Halbosiy *et al.* ., 2022). Numerous bioactive substances, including alkaloids, steroids, terpenoids, saponins, flavonoids, and phenolics, are present, demonstrating that this raw material is a medicinal plant, as suggested by Indriaty *et al.* . (2023).

Table 2. Initial detection of major active compounds in the different extracts and oil.

Test	Procedure	Aqueous	Ethanolic	Hexane
Glycosides	Benedict test Benedict reagent + water bath -ve blue +ve green to red	+++	++	+
Alkaloids	Mayer's test yellowish precipitate	+++	++	+
Phenols	Ferric chloride + bluish black	+++	++	+
Coumarin	Extract + concentrated NaOH +ve bright yellowish green	+++	++	++
Saponins	Heavy shake +ve long-lasting foam -ve clear solution	+++	++	+
Steroids, Terpenoids	Golden yellow Bright green color	++	++	++
Flavonoid	Ferric chloride -ve yellow +ve green	+++	++	+

‘‘+”indicates that the compounds are present;

"++" and "+++" show that a compound with a high concentration is present.

The concentration of peroxides is measured by the peroxide value (PV) and hydroperoxides that are formed during the early stages of oxidation in fats and oils. A higher peroxide value indicates degradation and potential health hazards.This procedure for storage provided the product with oxidative stability by the calculation of peroxide value. The influence of wheat germ on storage stability and its antioxidant component was examined during the investigation. From that list, oxidative stability should be the important criterion we need to analyze the inhibitory action of oil from wheat germ and extracts towards the process of oxidization in cream which takes place.To evaluate cream samples' storage stability, Wheat germ aqueous and ethanolic extracts were used in the analysis, as well as WGO, at concentrations of 0.2% and 0.4%.The data about peroxide values for cream samples from day 0 to 10 stored at 8 °C are displayed in Table 3. The cream samples were enriched with wheat germ aqueous, ethanolic extracts, and oil. When oil and extract results were contrasted, It was observed that cream samples prepared with WGO were effective in preserving the cream samples from oxidation during storage and determined the peroxide values for B1 and B2 were examined at 2.1699 and 2.5875 millimole per kilogram of fat, respectively, while the control value was 6.3719 millimoles per kilogramme of fat. The presence of bioactive compounds in the oil is responsible for this, which protects against oxidation. The protective effect of the oil in slowing down cream oxidation becomes more evident after 10 days of storage, as indicated in Table 3. Based on the findings (Table 3), is evident that the samples' peroxide levels with aqueous extract (C1, C2) increased after 10 days of storage to 7.5331 and 6.0992 mmol/kg. The rates of increase in PV of samples with aqueous extract were higher than the samples with ethanolic extract and oil.

The tocopherols in wheat germ ethanolic extract and oil may be responsible for their antioxidant properties (Brandolini and Hidalgo, 2012). According to several studies, antioxidant activity and

total phenolic content may be positively correlated (Labga *et al.* ., 2021). Antioxidants in food typically function as reducing agents, donating electrons and hydrogen ions to reverse oxidation. Natural antioxidants have received a lot of attention, and some highly active antioxidants that have been isolated from natural sources have been reported (Gulcin, **(**2020**)**.

The results of this investigation are consistent with those published by Soliman *et al.* . (2019). They observed that the peroxide value of free WGO increased rapidly when exposed to UV radiation, however, encapsulated WGO showed good oxidative stability after being exposed to UV light for up to 18 hours.

Table 3. Peroxide values of cream samples with wheat germ aqueous, ethanolic extracts and oil and storage at 8ºC for 0, 5 and 10 days.

No.	Treatments	Peroxide values		
		Zero-day	5 days	10 days
1	A	5.1090 ±0.63^{a}	3.7372±0.28 ^{a}	6.3719±0.59^{a}
2	B1	4.9631 ±0.49 ^{a}	2.3724 ±0.07^{b}	2.1699±0.18^{b}
3	B2	4.5713 ±0.41 ^{a}	4.7551±0.37 ^{b}	2.5875±0.14^{b}
4	C1	3.3405 ±0.27$^{b\ a}$	4.9883±0.41 ^{b}	7.5331±0.71^{bc}
5	C2	4.7179 ±0.52 ^{ac}	5.1055±0.57 ^{b}	6.0992 ±0.56^{d}
6	D1	2.9814 ±0.19 ^{a}	4.3602±0.42^{bc}	3.1981±0.33 ^{b}
7	D2	2.7692 ±0.08 ^{a}	3.1683±0.37 ^{b}	2.9672±0.19 ^{b}
L.S.D.		0.877 *	1.041 *	1.308 *

* (P≤0.05).

The data presented are the means of two replicate trials.
A= the control cream treatment, B1= adds 0.2% oil to the cream by weight, B2= adds 0.4% oil to the cream by weight, C1= 0.2% aqueous extract, C2= 0.4 % aqueous extract, D1= 0.2% ethanolic extract and D2= 0.4 % ethanolic extract.
Each column's values (mean ± SD, n = 2) that are superscripted with various lowercase letters differ significantly ($p < 0.05$).

Table 4 looks at FFA levels of cream samples with emulsifying agents, made based on wheat germ aqueous, ethanolic and oil extracts. FFA content is not a rare case, it one of the most used indicators that tell the extent of lipids' hydrolytic rancidity. The values of FFA in % wt. of oleic acid, for both B1 and B2 samples were in the range of 0.3862% to 0.3301%, as presented in Table 4. FFA contents of the cream sample (C1) were determined as 0.3917 % compared with control 0.4446 %. Therefore, longer residence times and lower temperatures are efficient ways to lower FFA (Wang and Johnson, 2001). The untreated sample (A) exhibited notably higher FFA content compared to the samples treated with wheat germ extracts and oil. Among the treated samples, The study's findings demonstrated that the cream sample containing ethanolic extract from wheat germ (D2) displayed greater effectiveness in reducing FFA values (0.3637%) compared to the other sample (D1) (0.6170%), although there was no statistically significant difference. In the present study, the obtained FFA are less than the previous reports resulted in Arslan *et al.* ., (2020) found the free fatty acid value of WGO was in the range of (2.7 - 5.5%).

Table 4. Free fatty Acids % of cream samples with wheat germ aqueous, ethanolic extracts and oil and storage in the cooling at 8ºC for 0, 5 and 10 days.

No.	Treatments	Oleic acid %		
		Zero-day	5 days	10 days
1	A	0.3851 ±0.08[a]	0.3919 ±0.10[a]	0.4446±0.13[a]
2	B1	0.3859 ±0.06 [a]	0.3862±0.08 [ab]	0.3975±0.09[b]
3	B2	0.4410 ±0.11 [a]	0.3301 ±0.08[c]	0.3015±0.06[c]
4	C1	0.5507 ±0.17 [ab]	0.2799 ±0.07 [a]	0.3917±0.09[c]
5	C2	0.4952 ±0.11[c]	0.4993 ±0.11 [a]	0.6088±0.18[c]
6	D1	0.4482 ±0.23 [a]	0.4970 ±0.16 [a]	0.6170±0.21[c]
7	D2	0.4955 ±0.19 [a]	0.3922 ±0.08[d]	0.3637±0.11[d]
	L.S.D.	0.126 *	0.142 *	0.175 *
		* (P≤0.05).		

The data presented are the means of two replicate trials.
A= The control cream
treatment, B1= adding 0.2% oil to the cream by weight, B2= adding 0.4% oil to the cream by weight, C1= 0.2% aqueous extract, C2= 0.4 % aqueous extract, D1= 0.2% ethanolic extract and D2= 0.4 % ethanolic extract.
Each column contains values (mean ± SD, n = 2) that are significantly ($p < 0.05$) different and are superscripted with different lowercase letters.

The findings of the sensory analysis performed on the various cream samples are shown in Table 5. Package shape (rated out of 10 points), public acceptance (rated out of 20 points), taste and flavor (rated out of 45 points), body and texture (rated out of 20 points), and color (rated out of 10) were among the sensory attributes used to evaluate the cream sample Significant and noticeable differences in taste and flavor were observed between the treated cream samples and the untreated control (A). As the concentration rose, the overall scores of cream samples with aqueous extract dropped (C1, C2). The cream samples having a concentration of essential oils of 0.4% (B2) were rated as the most acceptable. Furthermore, in every instance, as the samples were stored, the overall sensory evaluation scores steadily dropped A, C1, C2, and A1. After being subjected to cold storage for ten days, the public's acceptance somewhat declined. of cream samples from treatments A, C1, C2, and D1, as indicated in Table 5. The sensory evaluation revealed that cream samples B1, B2, and D2 received higher scores for public acceptance, body and texture (with B2 scoring higher than the control sample A), and color (with B1 and B2 scoring higher than the control sample A). On the other hand, cream samples prepared with aqueous and ethanolic extracts obtained reduced ratings for every sensory characteristic. Additionally, cream samples from therapies A, C1, C2, and D1 showed reduced ratings after the 10-day storage period. These findings corroborate those mentioned by (Soliman *et al.* ., 2019; Al-Rimawi *et al.* ., 2020).

Table 5. Sensory evaluation of the parameters of cream manufactured with the addition of wheat germ extracts and oil during a storage of ten days at a temperature of four degrees Celsius ±2*.

Treatment	Age of cream (days)	Package shape (5)	Public acceptance (20)	Taste and flavour (45)	Body and texture (20)	Colour (10)
A	0	5 ±0.67[a]	20 ±1.6 [a]	45 ±2.7 [a]	18 ±1.2[a]	9 ±0.61[a]
	5	5 ±0.67 [a]	20 ±1.6 [ab]	40 ±1.96[b]	15±0.76 [a]	8 ±0.57 [a]
	10	5 ±0.67 [a]	17 ±0.85 [a]	40 ±1.96 [b]	13±0.65 [a]	8 ±0.57 [a]
B1	0	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
	5	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	18 ±1.2 [a]	10 ±0.0 [a]
	10	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	18 ±1.2 [a]	10 ±0.0 [a]
B2	0	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
	5	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
	10	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
C1	0	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7[c]	18±1.2 [ab]	10 ±0.0 [ab]
	5	5 ±0.67 [a]	18 ±0.89 [a]	38 ±1.79 [b]	10±0.75 [a]	7 ±0.46 [a]
	10	5 ±0.67 [a]	18 ±0.89 [a]	38 ±1.79 [b]	9 ±0.63 [a]	6 ±0.37 [a]
C2	0	5 ±0.67 a	20 ±1.6 a	45 ±2.7 b	18 ±1.2 a	10 ±0.0 a
	5	5 ±0.67 a	18 ±0.89 a	38±1.79d b	15±0.78 a	9 ±0.61 a
	10	5 ±0.67 a	17 ±0.82 a	35 ±1.65 b	13±0.61 a	9 ±0.61 a
D1	0	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	18 ±1.2 [a]	10 ±0.0 [a]
	5	5 ±0.67 [a]	18 ±0.89 [a]	43 ±3.5 [b]	18 ±1.2 [a]	9 ±0.61 [a]
	10	5 ±0.67 [a]	18 ±0.89 [a]	42 ±2.0 [b]	17±0.85 [a]	8 ±0.57 [a]
D2	0	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
	5	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
	10	5 ±0.67 [a]	20 ±1.6 [a]	45 ±2.7 [b]	20 ±1.7 [a]	10 ±0.0 [a]
	LSD value	0.405 NS	2.961 *	4.155 *	4.927 *	2.071 *
			* = (P≤0.05)			

The data presented are the means of ten replicate trials.

Significant differences (p ≤0.05) exist between means in the same column that has different superscript letters A= the control cream treatment, B1= adding 0.2% oil to the cream by weight, B2= adding 0.4% oil to the cream by weight, C1= 0.2 % aqueous extract, C2= 0.4 % aqueous extract, D1= 0.2% ethanolic extract and D2= 0.4 % ethanolic extract.

Significant differences (p ≤0.05) exist between means in the same column that have different superscript letters.

This research provides the main principles for the creation of novel dairy products with functional properties and makes an indication that wheat germ extracts and oil can be a common product in the food industry because till now, it considerably lacked this type of product.

CONCLUSION

This work showed that in the case of applying wheat germ extracts and oil, it is possible to replace synthesis preservatives in food production. The study focused on the phytochemical analysis of the posed samples and oil using qualitative methods. We also found that water, ethyl-alcohol as well as oil, contained the highest density of phytochemicals. The obtained wheat germ extracts have exhibited good antioxidant activity. It can be used as a natural preservative, which requires a low dosage to maintain perfect condition. These supplements of extracts and oil will help manufacture an effective cream that has high antioxidant activities together with high quality that is well-known from the traditional cream. Oils and extracts that are hydrophilic and lipophilic can be combined with cream fat at concentrations of 0.2% and 0.4%. This formulation helps prolong the life of the cream up to 10 days at 8 ± 1°C while it remains nutritious to the consumers concerning taste, flavor, body, texture, and color. Furthermore, the fat replacer development involved adding a standardized dose of wheat germ extracts and oil that benefited the cream's physicochemical properties as well as organoleptic features.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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