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RESEARCH ARTICLE

Bioactive Compound and Fatty Acid Profile Analysis of Cold-pressed Flavor Oil

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ABSTRACT

Flavor oil is an innovative cooking oil mixed with herbs, spices, and fruit extract oils. This product innovation uses sunflower oil obtained through a cold press extraction method, which preserves the nutrients of the raw ingredients. In this study, there are four sunflower oil-based flavor oil variants: F1 (garlic oil), F2 (F1 + rosemary oil), F3 (F1 + red curry oil and chili), and F4 (lemon oil, mandarin orange oil, and pineapple oil). Fatty acid content was analyzed using a Chromatography Ionized Detector (GC-FID). In contrast, micronutrients (vitamins A, E, D3, and minerals) were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP - OES), Performance Liquid Chromatography - Photodiode Array (HPLC - PDA), and Liquid Chromatography-Tandem - Mass Spectrometry (LC-MS/MS). Research results show that all four products contain higher levels of unsaturated than saturated fatty acids. Oleic and linoleic acids dominate the composition of the unsaturated fatty acids. These products also contain vitamins A (32.81 - 1354.88 mcg/100g), E (16.49 - 30.55 mg/100g), and D3 (0 - 0.48 mcg/100g). Mineral content includes potassium (2.59 - 4.6 mg/100g), magnesium (0.29 - 0.44 mg/100g), zinc (0.06 - 0.12 mg/100g), and calcium (0.33 - 1.39 mg/100g). Based on literature studies, these products' fatty acids and micronutrients play a role in preventing cardiovascular diseases and high blood pressure, making them suitable as practical cooking oils that support a healthy lifestyle.

KEYWORDS: *Cold-pressed, Fatty acid, Flavor oil, Formulation, Health, Vitamin*

Introduction

Palm oil is derived from the oil palm tree (*Elais guineensis*) which represents the largest source of vegetable oil. This vegetable oil has the most balanced fatty acid composition but its saturated fatty acid makes a significant risk to Cardiovascular Diseases (CVD) (Chew *et al.*, 2022; Jiménez-Cortegana *et al.*, 2021). Consequently, several seed oils have emerged as alternatives to palm oil, driven by rising consumer awareness of the relationship between diet and health (Ali & Ali, 2020). One such alternative is sunflower oil. Sunflower oil (*Helianthus annuus*) has a lower saturated fat content; however, its high polyunsaturated fat relative to monounsaturated fat makes it more susceptible to oxidation. To maintain the quality of sunflower oil, producers commonly add synthetic antioxidants, such as butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) (Dunford, 2015). Nevertheless, BHT, TBHQ, and PG have negative effects on health rather than natural antioxidants derived from herbs or spices. The addition of these natural antioxidants also imparts a unique aroma and sensory of the product (Gonçalves-Filho & De Souza, 2022).

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Flavor oil is formulated with aromatic herbs or spices oil to impart flavors, aroma, and sensory to foods and offers some potential health benefits because of its bioactive and antioxidants. (Gonçalves-Filho & De Souza, 2022). Research by Meng *et al.* (2021) showed that sunflower oil infused with essential oil from Huai Chrysanthemum *x morifolium* Ramat. essential oil improved the antioxidant stability of sunflower oil. Based on p-anisidine and peroxide values, the performance of the highest concentration (1600 mg/kg) Huai Chrysanthemum *x morifolium* Ramat. essential oil was comparable to that of 200 mg/kg TBHQ, with a higher sensory score than oil with TBHQ. High content in MUFAs and omega-3 PUFAs, with the addition of the antioxidant phenolics will reduce the risk of heart disease by increasing HDL cholesterol, lowering LDL cholesterol, and preventing oxidative stress damage (Tian *et al.*, 2023). Cold-pressed oil has bioactive compounds along with omega-3s will modulate inflammatory pathways and the antioxidants will support cognitive health, reducing the risk of Alzheimer's disease and cognitive decline. Vitamin E and carotenoids bolster the immune system and protect skin from UV damage and premature aging (Meng *et al.*, 2021).

Therefore, the flavor oil industry has grown fast due to the high demand for natural sources of flavors and aromas. The innovations in flavor oils include flavors, aromas, and health trends. With increasing public awareness of the importance of choosing healthy foods and using natural ingredients with added benefits, flavored oils are becoming increasingly popular, especially among health-conscious consumers (Wandhekar *et al.*, 2023; Shahidi & Hossain, 2022). These oils contain antioxidants, vitamins, and essential fatty acids, that offer health benefits. By incorporating cold-pressed flavor oils into a diet, they can improve consumers' health without compromising culinary satisfaction (Meng *et al.*, 2021). This study aims to develop flavor oil innovations through four formulations and analyze the bioactive components of these four types of sunflower-based flavor oils based on literature studies to support their health benefits.

Materials and Method

Materials

Cold-pressed sunflower oil, garlic oil, rosemary oil, curry red oil, capsicum oil, lemon oil, pineapple oil, mandarin orange oil, EMSURE HNO_3, sodium, EMSURE KOH, IKA aquabides, ethanol, EMSURE glacial acetic acid, pyrogallol, EMSURE tetrahydrofuran, BF_3, methanol, EMSURE NaCl, EMSURE hexane, and anhydrous Na_2SO_4.

Instrumentation

Analytical balances, hot plate, water bath, magnetic stirrer, syringes (0.2 μm and 0.45 μm), automatic hydraulic press (Zhengzhou Bafang Machinery and Equipment Co., Ltd., Zhengzhou, China), nitrogen evaporator, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Agilent series 700, High-Performance Liquid Chromatography-Photodiode Array (HPLC-PDA), Gas Chromatography-Flame Ionized Detector (GC-FID) Agilent 7890B, and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

Extraction of Cold-Pressed Sunflower Oil

Based on the methods of Wandhekar *et al.* (2023), the extraction process begins with preparing raw sunflower seeds, which are cleaned, washed, and dried until the moisture content is below 7% for optimal extraction. The seeds are crushed using an automatic hydraulic press at 25 ± 1˚C with a pressure of 40 MPa for 30 minutes. After pressing, the oil is allowed to flow out for 5 minutes, then left to settle, forming sediment. The clear oil is filtered and stored in a dark container at room temperature for formulation.

Formulation of Cold-Pressed Flavor Oils

Four formulations (F1, F2, F3, and F4) were prepared by mixing sunflower oil with different spice oils. Formulation F1 combines sunflower oil with 25% garlic oil. Formulations F2 and F3 build on F1 by adding 20% rosemary oil and varying amounts of curry red and capsicum oils (2% and 1%, respectively). F4 combines sunflower oil with 6% lemon, 6% pineapple, and 6% mandarin orange oil. Each mixture is stirred at 300 rpm for 20 minutes at room temperature to achieve uniformity. The formulations and method for mixing were systematically assessed based on the aroma, flavor, and stability of its oil extracts as determined through researcher-led evaluation.

Table 1. Formulations for flavor oil production.

Component	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Sunflower Oil	75	71.25	70.5	82
Garlic Oil	25	23.75	23.5	0
Rosemary Oil	0	5	0	0
Red Curry Oil	0	0	4	0
Capsicum Oil	0	0	2	0
Lemon Oil	0	0	0	6
Pineapple Oil	0	0	0	6
Mandarin Orange Oil	0	0	0	6

Note: The amounts of ingredients are expressed as percentage volume per volume (v/v).

Mineral Content Analysis

Mineral content was determined using ICP-OES (Agilent 700), following methods by Giacomino *et al.* (2022). A sample (0.5 - 1 g) is treated with 10 ml of HNO_3 and digested with a microwave digester. It is then cooled and transferred to a 50 ml volumetric flask with yttrium as internal standard and aquabides as diluents. Then, the mixture was filtered using 0.2 µm syringe filter.

Vitamin A and E Analysis

Vitamin A and vitamin E content was analyzed following method by Köseoğlu *et al.* (2020) using HPLC with some modifications. As much as 2-3 grams sample was mixed with antioxidants, 95% ethanol, and 50% KOH then was heated at 80˚C for 45 minutes. After cooled down, the sample then transferred to 100 ml flask and diluted using tetrahydrofuran-ethanol solution and get filtered. The filtered sample was then injected into HPLC with RP-18 column. Last, the sample was detected using wavelength 325 nm for vitamin A and 290 nm for vitamin E.

Vitamin D3 Analysis

Vitamin D3 content was analyzed following method by Švarc *et al.* (2021) using LC-MS/MS with some modifications. As much as 1 gram of sample was homogenized with aquabidest, 95% ethanol, 50% KOH, and pyrogallol then get saponified in a waterbath. The sample then was cooled and evaporated until get the residue that filtered and injected the filtrate into LC-MS/MS with HSS T3 column with ammonium formate in methanol and aquabidest as the mobile phases of the sample.

Fatty Acid Analysis

Fatty acid content such monounsaturated fatty acid and polyunsaturated fatty acid were analyzed following method by Pandiangan *et al.* (2020) using GC-FID with some modifications. The sample is mixed with KOH-methanol solution, homogenized, heated, cooled, and treated with BF_3-methanol, followed by saturated NaCl in hexane. After phase separation, the organic layer is collected and analyzed on a Supelco SPTM 2560 column with nitrogen as the carrier gas. The chromatogram results are compared with reference spectra from the Wiley library.

Results and Discussion

Cold-pressed Flavor Oil Formulation and Usage

The flavor oil product formulation is developed using a blending method, which combines the carrier oil with various aromatic oils. The carrier oil in this flavor oil innovation is cold-pressed sunflower oil (Figure 1). The cold-press extraction method was chosen because it is simple, and does not require heat or chemicals. Heating can result in nutrient and bioactive compounds loss from the ingredients. The selection of spice oil variants in this product is based on preliminary research and researcher-led evaluations based on their flavor profile compatibility.

Figure 1. Cold-pressed sunflower oil (source: personal documentation).

According to Hashempour-Baltork *et al.* (2016), mixing vegetable fats/oils with similar properties is one of the simplest methods for creating a new product with specific texture and oxidative stability characteristics. Blending vegetable oils has been widely practiced to achieve the desired oil quality. Incorporating aromatic oils into vegetable oils has been found to increase their stability, and antioxidant activity, and prolong products' shelf life, for example, Kaseke *et al.* (2021) mixed sunflower oil with pomegranate seed oil, and Aly *et al.* (2021) mixed sunflower oil with moringa or sesame leaf oils.

Four formulations of flavor oil were created in this study. Blending oil with oil was done due to its similar physicochemical properties, allowing easier mixing process compared that water with oil. This process does not require chemical additives like emulsifiers, typically used in water-in-oil (W/O) or oil-in-water (O/W) mixtures. The cold-pressed flavor oil product is packaged using aerosol bottles. This product is expected to increase practical value and flexibility of use for consumers (Lin *et al.*, 2024). Aerosols are generally used for products that require foaming effects such as whipped cream and are applied as liquid sprays such as edible oils (Girardon, 2019). This aerosol spray bottle packaging uses aluminum monoblock. The choice of this material is based on its ability to be highly resistant to static pressure and explosion, recyclable, does not require lubricants during

the production process, and does not require intensive cleaning of its surface (Niemiec *et al.*, 2018). Additionally, aluminum packaging also protects products from light and air, protecting flavor oil from oxidation.

Aerosols work on the principle of a plastic dip tube connected to a valve, allowing liquid to move from the bottom of the can to the outside due to gas pressure. This pressure is generated by nitrogen through a compressed gas system, where nitrogen gas is pumped into the container through a high-pressure valve after the liquid product has been inserted and sealed. This pressure gives the liquid product a strong push so that it can be ejected properly through a nozzle (Girardon, 2019). This nozzle will serve to atomize the liquid into small droplets and form a fine spray (Girardon, 2019). This fine spray causes the molecules released to be small so that they can absorb more easily in the food being processed. This innovation is promising but also required to ensure product compatibility with the aerosol mechanism by checking its formulation to prevent clogging and oil degradation.

This flavor oil also has been certified by the Food and Drug Administration (BPOM) which verifies the safety and benefits of the product and has also received Halal certification which indicates that the product has undergone inspection and meets the principles and laws of Islamic sharia, especially in terms of hygiene, safety, and halalness of the ingredients used. The following certifications also increase the credibility of the quality and ensure the hygiene of the product (Aslan, 2023).

Vitamin and Mineral Content in Flavor Oil

Spice oil addition can alter the nutrient composition of flavor oil, as shown in Table 2 and Table 3, the vitamin and mineral content of each flavor are different. The addition of garlic oil in the F1 formulation did not increase the vitamin A and D3 content, unlike formulations F2-F4, which included other spice oils. Vitamins A and E act as antioxidants, preventing oxidative stress and boosting immunity (Carazo *et al.*, 2021; Rizvi *et al.*, 2014). Additionally, the F3 formulation showed the highest vitamin E content. Regarding mineral content, garlic oil was identified as the primary source of calcium, as F1, with the highest garlic oil content, yielded higher calcium levels than the other three formulations. Calcium induces lower cholesterol by increasing HDL and reducing LDL, with the help of vitamin D3 which influences calcium concentration in the body (Jensen *et al.*, 2023; Cormick, 2019). Similarly, formulation F3 also had the highest levels of the other three analyzed minerals. Based on the USDA database, the ingredients' raw material nutrients such rosemary, curry leaves, and mandarin oranges contain vitamin A, while garlic does not and have a higher content of calcium. Surprisingly raw rosemary has a higher calcium content compared to garlic, however, based on the result the addition of rosemary oil did not increase the calcium content of the formulated oil, this could be due to the extraction method that was unable to extract the calcium in rosemary. F1-F4 contains four key minerals; calcium, magnesium, potassium, and zinc that enhances the nutritional value (Wabo *et al.*, 2022; Weyh *et al.*,2022). In variant F3, higher potassium and magnesium contents were found compared to the other three variants. This can be caused by the addition of red curry extract. Based on research Pathak & Singh, 2022, it was found that curry leaves have a magnesium and potassium content of 147.11 ± 0.052 and 427.35 ± 0.35 mg/100g.

Table 2. Vitamin content in flavor oil products.

Parameters	Vitamin Content (mcg/100g)			
	F1	F2	F3	F4
Vitamin A	32.81	1354.88	1354.12	1354.79
Vitamin E	16.60	16.49	30.55	24.7

Vitamin D3	-	0.44	0.48	0.45

Note:
F1: Sunflower Oil + Garlic Oil
F2: F1 + Rosemary Oil
F3: F1 + Red Curry Oil + Capsicum Oil
F4: Sunflower Oil + Lemon Oil + Mandarin Orange Oil + Pineapple Oil

Table 3. Mineral content in flavor oil products.

Parameters	Mineral Content (mcg/100g)			
	F1	F2	F3	F4
Potassium	2.77	2.97	4.60	2.59
Magnesium	0.36	0.29	0.44	0.31
Zinc	0.11	0.10	0.12	0.06
Calcium	1.39	0.90	0.87	0.33

Notes:
F1: Sunflower Oil + Garlic Oil
F2: F1 + Rosemary Oil
F3: F1 + Red Curry Oil + Capsicum Oil
F4: Sunflower Oil + Lemon Oil + Mandarin Orange Oil + Pineapple Oil

Fatty Acid Content

Sunflower oil primarily contains unsaturated fatty acids, with oleic acid (C18:1) and linoleic acid (C18:2) being the dominant types at levels of 45.4% and 40.1%, respectively (Patil & Singh, 2022). The fatty acid content of each flavor oil is shown on Table 4. The results align with the statement above, which states that all flavor oils using cold-press sunflower oil have dominant unsaturated fatty acid. The highest unsaturated fatty acid was found on variant 4 that used lemon, mandarin, and pineapple oil, while the lowest was found on variant 1 that used only garlic oil as the addition. The higher the content of unsaturated fatty acid can offer more health benefits compared to higher saturated fatty acid, which is often linked to several non-communicable diseases (Mercola & D'adamo, 2023; Thomas *et al.*, 2020). However, unsaturated fatty acid structure is less stable compared to saturated fatty acid, making it susceptible to oxidation and degradation.

A study by Patil & Singh (2023) found that the quality of sunflower oil when used for deep-frying fried foods was lower than that of palm oil. The quality of sunflower oil can be seen from the % free fatty acids (FFA) and peroxide value (PV) which are 1.5-3.5 and 1.45-18 times higher than palm oil. However, because the main function of flavor oil is as a shallow frying oil with a lower temperature than deep frying, the damage to oil quality is lesst (Lima *et al.,* 2024). In addition, the addition of spice oil also acts as a source of natural antioxidants for the product which aims to maintain the quality of the oil during the storage process.

Table 4. Fatty acid composition.

Fatty Acid	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Saturated Fatty Acids	**46.72**	**31.18**	**30.22**	**25.41**
Caproic Acid (C6:0)	0.28	0.09	0.11	10.97
Caprylic Acid (C8:0)	4.35	1.73	1.66	0.42
Capric Acid (C10:0)	3.28	1.41	-	0.29
Undecanoic Acid (C11:0)	-	-	0.01	0.04
Lauric Acid (C12:0)	23.12	11.75	11.28	2.71
Tridecanoic Acid (C13:0)	-	0.04	0.04	0.06
Myristic Acid (C14:0)	6.38	4.90	4.73	1.18

Pentadecanoic Acid (C15:0)	-	0.014	0.016	0.012
Palmitic Acid (C16:0)	6.56	7.59	8.73	5.89
Heptadecanoic Acid (C17:0)	-	0.0403	0.0444	0.03
Stearic Acid (C18:0)	2.09	2.89	2.90	2.86
Arachidic Acid (C20:0)	0.13	0.17	0.17	0.21
Behenic Acid (C22:0)	0.36	0.38	0.36	0.55
Lignoceric Acid (C24:0)	0.12	0.14	0.13	0.19
Unsaturated Fatty Acids	**53.17**	**68.70**	**69.66**	**74.51**
Myristoleic Acid (C14:1)	-	0.08	0.01	-
Palmitoleic Acid (C16:1)	0.12	0.16	0.17	0.11
Heptadecenoic Acid (C17:1)	-	0.04	0.05	0.03
Oleic Acid (C18:1)	25.11	34.77	36.74	31.03
Linoleic Acid (C18:2)	27.85	33.58	32.50	43.16
Linolenic Acid (C18:3)	-	0.03	0.06	0.04
Eicosenoic Acid (C20:1)	-	0.10	0.11	0.12

Notes:
F1: Sunflower Oil + Garlic Oil
F2: F1 + Rosemary Oil
F3: F1 + Red Curry Oil + Capsicum Oil
F4: Sunflower Oil + Lemon Oil + Mandarin Orange Oil + Pineapple Oil

Conclusion

Formulations 2, 3, and 4 have higher vitamins A, E, and D3 rather than formulation 1 because of rosemary, red curry, capsicum, lemon, pineapple, and mandarin orange oil as addition in F2, F3, and F4. Among 4 formulations, F3 has higher calcium, magnesium, potassium, and zinc due to its red curry oil. Moreover, flavor oils also contain a lower percentage of saturated fats (25.41% - 46.73%) than unsaturated fats (53.17% - 74.51%), resulting in a healthier option of oil for heart health benefits and possess anti-inflammatory properties. Based on the flavor profiles by researchers' personal evaluation, F1 can be used to base seasoning for various dishes, F2 has a minty and woody flavor for meat-kind of dishes, F3 provides a mild flavor for neutralizing fishy taste in seafood dishes, and F4 with the fruity fresh and vibrant flavor that perfect use as a salad dressing.

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Conflict of Interest

We declare in this work, there are no conflict of interest.

Author Contributions

AYDP conducted the experiment and analyzed the data, JASHP conducted the experiment and wrote the manuscript, YASP wrote the manuscript, L supervised the experiment, and DW supervised the experiment and designed the research. All authors agreed to the final version of this manuscript.

Ethical Statement

The ethical statement was not required for this study. This article does not contain any studies involving animals performed by any of the authors.

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